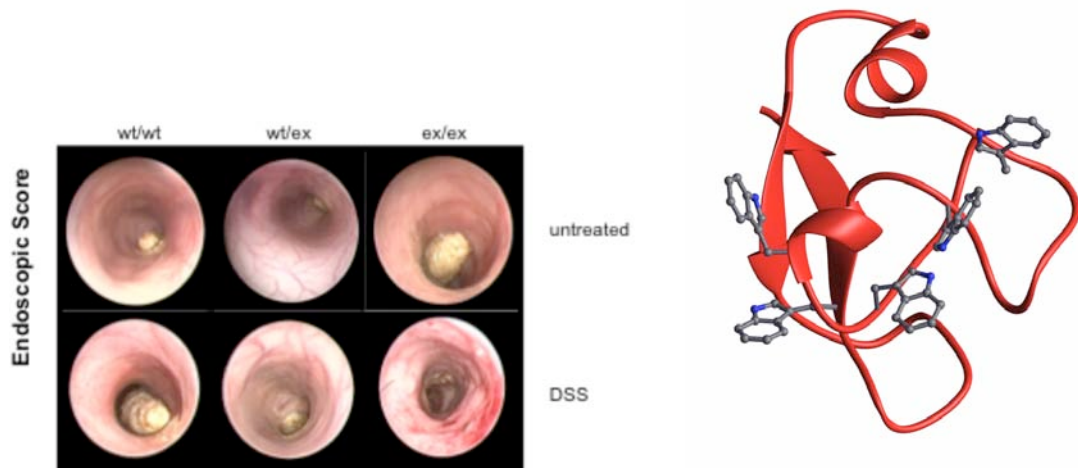
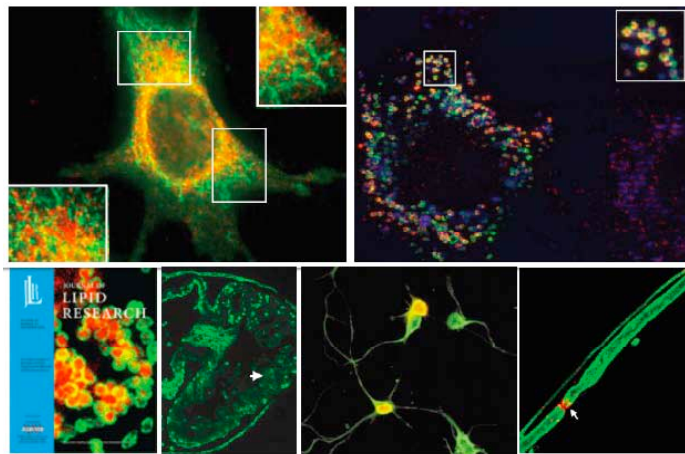


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Biochemical Institute

Christian-Albrechts-University of Kiel

Research Report 2008/2009



Contens

Scientific Staff Members 2008/2009.....	4
Prof. Dr. rer. nat. Stefan Rose-John – Executive Director	4
Prof. Dr. rer. nat. Paul Saftig – Director	4
Prof. Dr. med. Ursula Just – Director.....	4
Prof. Dr. med. Roland Schauer – Emeritus	4
Post-Docs:	4
Doctoral students:.....	4
MD Students:.....	5
Contact	6
Editorial	7
1. Research Group Prof. Dr. Stefan Rose-John	8
2. Research Group Prof. Dr. Hilmar Lemke.....	13
3. Research Group Prof. Dr. Joachim Grötzinger	15
4. Research Group Prof. Dr. Jürgen Scheller	19
5. Research Group Prof. Dr. Paul Saftig	23
6. Research Group Prof. Dr. Karina Reiss.....	32
7. Research Group PD Dr. Michael Schwake	35
8. Research Group Dr. Judith Blanz	37
9. Research Group Dr. Bernd Schröder.....	41
10. Research Group Prof. Dr. Ursula Just	44
11. Research Group Dr. Thomas Höfken	48
12. Research Group Dr. Ralf Schwanbeck.....	50
13. Research Group Prof. Dr. Roland Schauer.....	52
Seminars 2008/2009.....	57
Publications 2008/2009	59
Accumulated Impact Factors.....	65

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Editorial

The positive feedback we received after our three preceding bi-annual reports has encouraged us to now publish our fourth report for the years 2008/2009. After the Institute of Biochemistry at the Christian-Albrechts-Universität, Kiel has undergone fundamental changes in the years 2000-2002, we are now in a phase of consolidation and expansion. Renovations in the Institute are close to completion and all the laboratories and teaching areas of the institute have been fully modernized.

In the years 2008 and 2009, members of the Biochemistry Institute were represented in all three biomedical collaborative research centers (SFB415: "Specificity and Pathophysiology of Signal Transduction Pathways", SFB617, "Molecular mechanisms of epithelial defense" and SFB654: "Plasticity and Sleep"), in which the Christian-Albrechts-University of Kiel is involved. Late in 2009, a new SFB841 "Liver inflammation: infection, immune regulation und consequences" was approved by the Deutsche Forschungsgemeinschaft and is going to start in January 2010. This new SFB is coordinated by the University Hospital Hamburg-Eppendorf with participation of the Biochemistry Institute in Kiel.

Since the SFB415 will end in summer 2010, the Biochemistry Institute has taken the initiative to apply for a new SFB with the title 'Proteolysis as a regulatory event in pathophysiology'. This initiative was pre-reviewed by a board of the Deutsche Forschungsgemeinschaft in 2009 and we were invited to prepare a full proposal. Our initiative will be visited by a reviewing panel of the Deutsche Forschungsgemeinschaft in March 2010.

Furthermore, the scientific work of members of the Institute of Biochemistry is supported by additional grants from the Deutsche Forschungsgemeinschaft and the European Union.

Since 2008, the cluster of excellence 'Inflammation at Interfaces' formed by scientists from the University of Kiel, the University zu Lübeck and the Research Center in Borstel is funded for five years. Scientists from the Biochemistry Institute are prominently involved in the cluster of excellence and one of the three major research projects of the cluster is coordinated at our Institute. Consequently, a new junior research group and a new professorship have been established at the Institute of Biochemistry.

Thus, our Institute continues to represent a spearhead in biomedical research in the Northern Germany landscape of science. In this brochure you can find an overview of the projects being carried out in the Institute of Biochemistry in 2008 and 2009, along with internet addresses where you can find more detailed information.

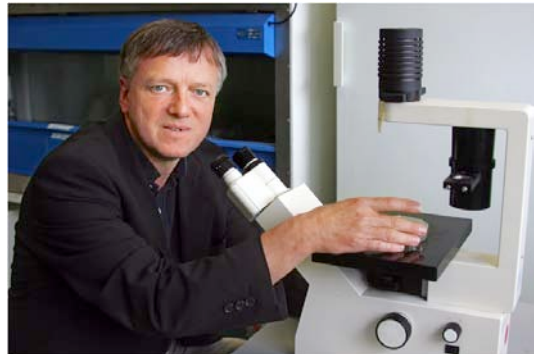
Kiel, January 2010

Stefan Rose-John

1. Research Group Prof. Dr. Stefan Rose-John

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Prof. Dr. Stefan Rose-John



B Lab Members:

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Dr. Stephanie Tenhumberg
Dr. Claudia Drucker
Dr. Athena Chalaris
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Christin Dewitz
Christoph Garbers
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Sven Malchow
Ulrike May
Antje Schütt
Jan Suthaus
Ulrike May

Technicians:

Stephanie Schnell
Michaela Jahn
Inez Götting

C Research Report

C.1. The soluble interleukin-6 receptor: generation and physiologic function

The soluble interleukin-6 receptor (IL-6R) in complex with interleukin-6 (IL-6) stimulates cells, which express the signaling receptor subunit gp130 but no ligand binding IL-6R. Such cells in the absence of the soluble IL-6R are unable to respond to IL-6. This process has been named 'trans-signaling'. Trans-signaling has been shown to be important for inflammation reactions, neuronal survival, hematopoiesis and tumor rejection.

We have characterized the metalloproteinase, which is responsible for the release of the soluble IL-6R by biochemical and genetic means. This protease belongs to the Metalloproteinase with Disintegrin Domain (ADAM) family of metalloproteinases. We plan to perform experiments to better understand the biochemical and structural prerequisites of limited proteolysis of the IL-6R by members of the ADAM family. We also want to understand the regulation of the cleavage reaction. In this respect it is interesting that we could recently show that the induction of apoptosis leads to activation of the shedding protease ADAM17 (see below). This seems to

be a general phenomenon, which might play an important role in the regulation of the inflammatory process. We could show that neutrophils, which are the first line of defense of the body during infection and inflammation, are a major source of the soluble IL-6R *in vivo*. Interestingly, induction of apoptosis leads to a selective activation of ADAM17, which in turn is responsible for shedding of the IL-6R.

As mentioned above, we could show that the protease ADAM-17 (also called TACE), which is responsible for cleavage of TNF α is also strongly involved in shedding of the IL-6R. Using a novel homologous recombination strategy, we have recently constructed ADAM17 hypomorphic mice (called ADAM17^{ex/ex} mice) to analyze the involvement of this protease in various shedding processes. Using these ADAM17^{ex/ex} mice we currently study the physiologic role of cleavage of proteins of the ligands of the EGF-Receptor and of the notch protein.

The phenotype of the ADAM17 hypomorphic mice clearly shows an involvement of ADAM17 in inflammatory processes. Therefore these mice are an excellent experimental tool to study the overall physiologic role of the ADAM17 metalloprotease and its involvement in inflammation and cancer.

C.2. Viral Interleukin-6: Structure, Pathophysiology and Strategies of Neutralisation

On target cell Interleukin-6 (IL-6) binds to a receptor complex consisting of the ligand binding subunit Interleukin-6-receptor (IL-6R) and the signal transducing subunit gp130. The complex of soluble IL-6R and IL-6 acts on cells, which do not express IL-6R. Such cells would not be able to respond to IL-6 alone. Such cells comprise hematopoietic progenitor cells, endothelial cells, smooth muscle cells, T-cells and neural cells. Interestingly, the recently identified viral IL-6 (vIL-6) encoded by Human Herpes Virus 8 (HHV8) binds to and activates gp130 directly. Such, vIL-6 activates a significant larger spectrum of target cells than human IL-6. We want to characterize the biochemical and physiological properties of vIL-6. Furthermore, we are in the process of generating transgenic mice, which overexpress vIL-6. Using these mice we currently evaluate the involvement of vIL-6 in human diseases like Castleman disease, primary effusion lymphoma and multiple myeloma.

Using a novel strategy, we used our recently generated recombinant antibodies against vIL-6 to target the vIL-6 within cells expressing the protein. The underlying principle of this strategy is to anchor the recombinant vIL-6 antibodies within the endoplasmic reticulum (ER) with the help of the canonical ER retention sequence KDEL. Indeed we could demonstrate that vIL-6 can induce signaling from within the cell and that such signaling can be completely blocked from within the cell.

We have performed structure-function analysis to clarify how vIL-6 can bind directly to gp130 whereas human IL-6 needs the IL-6R in order to bind to and activate gp130. These data clearly show that the so-called site III of vIL-6 is responsible for this property and that the ability to directly bind to gp130 can be transferred to human IL-6 by transferring site III.

Transgenic mice, which over-express the vIL-6 protein show a phenotype resembling human Castleman disease. These data indicate that vIL-6 is strongly involved in the pathophysiology of HHV8.

C.3. Development of the IL-6 trans-signaling antagonist sgp130Fc

We could show in the past years that IL-6 trans-signaling can specifically be inhibited by the sgp130Fc protein without affecting IL-6 signaling via the membrane bound IL-6R. These data established the sgp130Fc protein not only as a molecular tool to experimentally distinguish between classic- and trans-signaling. The sgp130Fc protein can also be used to block the course of inflammatory diseases in animal models of rheumatoid arthritis, peritonitis, inflammatory bowel disease and inflammation induced colon cancer.

One goal of the next years will be to improve the properties of the sgp130Fc protein in terms of protein stability, affinity towards the IL-6/sIL-6R complex and feasibility of production. Furthermore, we are also engaged in a study to solve the three dimensional structure of the sgp130Fc bound to the IL-6/sIL-6R complex.

Since the sgp130Fc protein has a considerable therapeutic potential, Stefan Rose-John together with Prof. Stefan Schreiber (Director of the Institute of Clinical Molecular Biology at the University Hospital in Kiel) founded a biotechnology company (Conaris Research Institute), which develops the sgp130Fc protein into a drug. In December 2008, Conaris Research Institute and the company Ferring have completed an exclusive worldwide license agreement for the development of sgp130Fc for inflammatory conditions such as IBD and rheumatoid arthritis. Sgp130Fc is undergoing pre-clinical testing prior to moving in to Phase I in 2010.

D Publications 2008/2009

Publikationen 2008	Impact Factor
1. Linker LA, Lühder F, Kallen K-J, Lee D-H, Engelhardt B, Rose-John S and Gold R (2008) IL-6 transsignalling modulates the early effector phase of EAE and targets the blood-brain-barrier. <i>J Neuroimmunol</i> , 205 : 64-72.	3.159
2. Tenhumberg S, Waetzig GH, Chalaris A, Rabe B, Seegert D, Scheller J, Rose-John S and Grötzinger J (2008) Structure guided optimization of the IL-6 transsignaling antagonist sgp130Fc. <i>J Biol Chem</i> . 283 : 27200-27207	5,520
3. Weigmann B, Lehr HA, Yancopoulos G, Valenzuela D, Murphy A, Stevens S, Schmidt J, Galle PR, Rose-John S, and Neurath MF (2008) The transcription factor NFATc2 controls IL-6-dependent T cell activation in experimental colitis. <i>J Exp Med</i> . 205 : 2099-2110	15.219
4. Von Bismarck P, Claass A, Schickor C, Krause MF and Rose-John S (2008) Altered pulmonary interleukin-6 signaling in preterm infants developing bronchopulmonary dysplasia. <i>Exp Lung Res</i> . 34 : 694-706	1.618
5. West MA, Prescott AR, Chan KM, Zhou Z, Rose-John S, Scheller J, and Watts C (2008) TLR-ligand induced podosome disassembly is ADAM17 dependent. <i>J Cell Biol</i> . 182 : 993-1005	9.120
6. Mudter J, Amoussina L, Schenk M, Bruestle A, Weigmann B, Yu J, Wirtz S, Biesterfeld S, Galle PR, Lehr HA, Rose-John S, Mueller C, Lohoff M, and Neurath MF (2008) The transcription factor Interferon regulatory factor-4 controls experimental colitis via T cell derived interleukin-6. <i>J Clin Invest</i> . 118 : 2415-2426	16.559
7. Sudarman E, Bollati M, Hafner M, Müller W, Scheller J, Rose-John S and Eichler J (2008) Synthetic Mimetics of the gp130-Binding Site for Viral Interleukin-6 as Inhibitors of the vIL-6 - gp130 Interaction. <i>Chem Biol Drug Des</i> , 71 : 494-500	2.375
8. Marino M, Scuderi F, Provenzano C, Scheller J, Rose-John S, and Bartocioni E (2008) IL-6 regulates MCP-1, ICAM-1 and IL-6 expression in human myoblasts. <i>J Neuroimmunol</i> . 196 : 41-48	3.159
9. Benrick A, Jirholt P, Wernstedt I, Gustafsson M, Scheller J, Eriksson A-L, Borén J, Hedner T, Ohlsson C, Härd T, Rose-John S, Jansson J-O (2008) A non-conservative polymorphism in the IL-6 signal transducer (IL6ST)/gp130 is associated with myocardial infarction in a hypertensive population. <i>Regul Peptides</i> 146 : 189-196	2.276
10. Rabe B, Chalaris A, May U, Waetzig GH, Seegert D, Williams AS, Jones S, Rose-John S, Scheller J (2008) Transgenic blockade of Interleukin-6-transsignaling abrogates inflammation. <i>Blood</i> 111 : 1021-1028	10.432
11. Hieronymus T, Ruau D, Ober-Blöbaum J, Baek J-H, Rolletschek A, Rose-John S, Wobus AM, Müller AM, and Zenke M (2008) The transcription factor repertoire of Flt3+ hematopoietic stem cells. <i>Cells Tissues Organs</i> . 188 : 103-115	2.376
12. Walker F, Zhang H-H, Matthews V, Weinstock J, Nice EC, Ernst M, Rose-John S and Burgess AW (2008) IL6/sIL6R complex amplifies emergency granulopoietic response in the absence of G-CSF and GM-CSF. <i>Blood</i> , 111 : 3978-85	10.432
13. Nechemia-Arbely Y, Barkan D, Pizov G, Shriki A, Rose-John S, Galun E and Axelrod JH (2008) IL-6/IL-6R axis plays a critical role in acute kidney injury. <i>J Am Soc Nephrol</i> . 19 : 1106-1115	7.505
Publications 2009	Impact Factor
1. Sina C, Gavrilova O, Förster M, Till, A, Derer S, Hildebrand F, Rabe B, Scheller J, Chalaris A, Scheller J, Rehmann A, Franke A, Ott S, Häsler R, Nikolaus S, Fölsch UR, Rose-John S, Jiang H-P, Li J, Schreiber S and Rosenstiel P (2009) G-protein coupled receptor 43 (GPR43) is essential for neutrophil recruitment during intestinal inflammation. <i>J Immunol</i>	6.000

2. Drucker C, Gewiese J, Malchow S, Scheller J, and Rose-John S (2009) The impact of Interleukin-6 classic- and trans-signaling on liver damage and regeneration. <i>J Autoimmun</i> , in press	7.881
3. Hösel M, Quasdorff M, Webb D, Zedler U, Esser K, Arzberger S, Wiegmann K, Kirschning K, Langenkamp A, Rose-John S and Protzer U (2009) Not interferon, but IL-6 controls early gene expression in Hepatitis B virus (HBV) infection. <i>Hepatology</i>	11.355
4. Andratsch M, Mair N, Constantin CE, Scherbakov N, Benetti C, Vogl C, Sailer CA, Üceyler N, Brockhaus J, Martini R, Sommer C, Zeilhofer HU, Müller W, Kuner R, Davis JB, Rose-John S, Kress M (2009) Reversal of Cancer Pain through Anti-Interleukin-6 Treatment. <i>J Neurosci</i>	7.452
5. Benedict C, Scheller J, Rose-John S, Born S and Marshall L (2009) Enhancing Influence of Intranasal Interleukin-6 on Slow Wave Activity and Memory Consolidation During Sleep. <i>FASEB J</i> 23:3629-36	7.049
6. Drucker C, Rabe, Chalaris A, Schulz E, Scheller J, Rose-John S (2009) Interleukin-6 Trans-Signaling regulates glycogen consumption after D-Galactosamine induced liver damage. <i>J Interf Cyt Res</i>	1.774
7. May U, Schiffelholz T, Baier PC, Krueger JM, Rose-John S and Scheller J (2009) IL6-trans-signalling increases rapid-eye-movement sleep in rats. <i>Eur J Pharmacol</i> 613:141-145	2.787
8. Plagmann I, Chalaris A, Kruglov AA; Nedospasov S, Rosenstiel P, Stefan Rose-John S, Scheller J (2009) Transglutaminase-catalyzed covalent multimerization of camelidae anti-human TNF single domain antibodies improves neutralizing activity. <i>J Biotechnol</i> 142:170-178	2.748
9. Adam N, Rabe B, Suthaus J, Grötzinger J, Rose-John S and Scheller J (2009) Understanding receptor independent gp130 activation of viral interleukin-6. <i>J Virol</i> 83: 5117-5126	5.308
10. Nowell MA, Williams AS, Carty SA, Scheller J, Hayes AJ, Jones GW, Richards PJ, Slinn S, Ernst M, Jenkins BJ, Topley N, Rose-John S and Jones SA (2009) Therapeutic targeting of IL-6 trans-signaling counteracts STAT3 control of the inflammatory infiltrate in experimental arthritis. <i>J Immunol</i> , 182: 613-622	6.000
11. Chen L, Frister A, Wang S, Ludwig A, Behr H, Pippig S, Li B, Simm A, Hofmann B, Pilowski C, Koch S, Buerke M, Rose-John S, Werdan K, and Loppnow H (2009) Interaction of vascular smooth muscle cells and monocytes by soluble factors synergistically enhances interleukin-6 and MCP-1 production. <i>Am J Physiol-Heart C</i> . 296: H987-996	3.643
12. Baier PC, May U, Scheller J, Rose-John S, Schiffelholz T (2009) Impaired hippocampus-dependent and -independent learning in IL-6 deficient mice. <i>Behav Brain Res</i> . 200: 192-196	3.171
13. Grivennikov S, Terzic J, Karin E, Mucida D, Yu G-Y, Vallabhapurapu S, Rose-John S, Cheroutre H, Eckmann L and Karin M (2009) IL-6 and STAT3 signaling is required for survival of intestinal epithelial cells and colitis associated cancer. <i>Cancer Cell</i> , 15: 103-113	24.962
14. Islam O, Gong AX, Rose-John S and Heese K (2009) Interleukin-6 and Neural Stem Cells - More Than Gliogenesis. <i>Mol Biol Cell</i> , 20: 188-199	5.558

Impact factors 2008: 89.750

Impact factors 2009: 95.688

Total impact factors 2008/2009: 185.438

E Grants

- E.1 Stefan Rose-John
 Die Rolle von gp130-Trans-Signaling bei der Leber-Regeneration und -Krebs: therapeutische Perspektiven
 DFG RO 632/13-1, Fördersumme (2006 – 2010) 261.400 €

Research Report Biochemical Institute, CAU Kiel 2008/2009

- E.2 Jürgen Scheller und Stefan Rose-John
Der lösliche Interleukin-6-Rezeptor: Entstehung und physiologische Bedeutungen
Sonderforschungsbereich 415, Teilprojekt B5, Fördersumme (2007 – 2010) 248.700 €
- E.3 Stefan Rose-John
Virales Interleukin-6: Struktur, Pathophysiologie und Strategien zu seiner Neutralisierung
Sonderforschungsbereich 415, Teilprojekt C6, Fördersumme (2007 – 2010) 268.800 €
- E.4 Stefan Rose-John, Jürgen Scheller, Jan Born, Dunja Hinze-Selch
The function of the gp130-signaling-family for sleep and plasticity
Sonderforschungsbereich 654, Teilprojekt C5, Fördersumme (2005 – 2009) 444.600 €
- E.5 Stefan Rose-John and Jürgen Scheller
The function of the gp130-signaling-family for sleep and plasticity
Sonderforschungsbereich 654, Teilprojekt C5, Fördersumme (2009 – 2013) 444.600 €
- E.5. Exzellenzcluster Inflammation at Interfaces, Integrated Research Network F: gp130 signalling,
Fördersumme (2008-2012) 2.749.800 €; Miniproposal (2009): 50.000 €
- E.6. Exzellenzcluster Inflammation at Interfaces, Integrated Research Network F: gp130 signalling,
Fördersumme (2008-2012) 2.749.800 €; Miniproposal (2009): 50.000 €

2. Research Group Prof. Dr. Hilmar Lemke

A Group Leader:

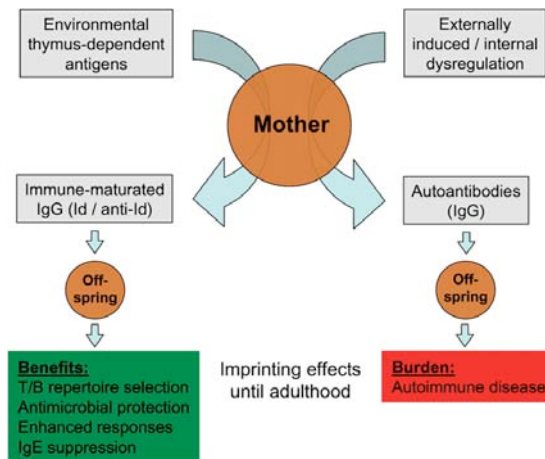
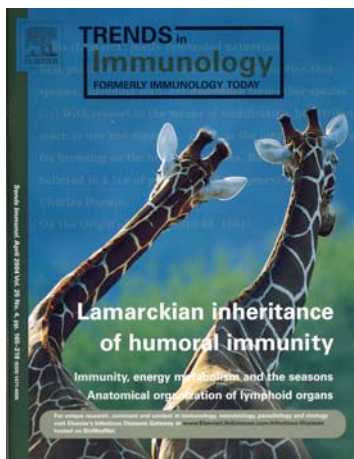
Prof. Dr. Hilmar Lemke



B Lab Members:

Post-Doc

Dr. rer. nat. Ahmad Trad



C Research Report

C.1. The cells of the adaptive immune system, B and T lymphocytes, with their clonally distributed receptors form a web of interacting variable domains, known as the *idiotypic network*. This exerts 2 functions. First, antigen-induced cellular activations by idiotypic cross-reactivity enable a generalization of single antigenic experiences so that the immune systems in its entirety benefits for its battle with environmental microbes. Second, idiotypic network interactions allow the transfer of the *collective maternal immunological experience* to the offspring thus enabling a maternally mediated *immunological education* before the first encounter with external antigens.

In the latter context, it was a remarkable finding that maternally-derived immune or monoclonal IgG antibodies (idiotypes) reactive with suitable model allergens like ovalbumin or bee venom phospholipase A₂ (bvPLA₂) are able to induce long-lasting IgE unresponsiveness in the offspring. Our latest investigations have shown that anti-idiotypic antibodies to the transgenerational IgE-suppressive IgG are likewise able to induce such IgE suppression. Since the effective anti-idiotype did not mimic epitopes of the allergen (bvPLA₂), we conclude that idiotypic interactions are mechanistically responsible to the transgenerational IgE-suppression by maternal IgG antibodies. These results may offer new ways for a prophylactic and possibly therapeutic treatment of human IgE-mediated allergies.

C.2. In this project, we analyze the model immune response to the hapten 2-phnel-oxazolone (phOx) and obtained some interesting results which are of general importance.

1.) The analysis of the thymus-dependent (TD) anti-phOx response following a pre-immunization with the thymus-independent type 2 (TI-2) form of the antigen demonstrated that the latter, in contrast to current understanding, induces a special sort of immunological memory which we named *network memory*. In addition, evi-

dence was obtained for the first time that receptor revision by VH replacement may occur during immune maturation in genetically non-engineered wildtype mice.

2.) We compared the phOx-specific repertoires of `natural` antibodies, *i.e.* phOx-reactive antibodies from non-immunized mice, with those of TI-2-induced primary and TD-induced primary, secondary, tertiary and quaternary antibodies. The analysis these IgM and IgG antibodies revealed that a) `natural`, TI-2- and TD-induced IgM antibodies used a huge repertoire of VH/VL genes, b) a restriction of the repertoire to a few V genes was first observed after class switch recombination to IgG, and c) IgM antibodies encoded by genes of the VH1 family hardly ever switched to IgG (Lange et al, in preparation).

3.) In addition, the complete anti-phOx IgM/IgG repertoire allowed an analysis of the amino acid sequences of the most variable part of antibodies, the third hypervariable region, VHCDR3. It has been concluded that the extreme variability of this region is sufficient to generate a full antibody repertoire, even when only 1 VH and 1 VL gene are available (Xu & Davis, 2000, Immunity 13: 37-45). In this report, antibodies with a particular specificity exhibited similar VHCDR3 sequences. In contrast, anti-phOx antibodies of our analysis did not show similar or identical motives in VHCDR3 (Lange et al, in preparation).

C.3. It is assumed that the VHCDR3 has 2 functions: on the one hand, it is of particular importance for binding of the epitope and, on the other, it is the primary target for idiotypic regulation, not only in B but also in T cells with their analogous V β CDR3. However, the significance of VHCDR3 for the regulation of an immune response is not known. Previous results had indicated that there might be a correlation between immune maturation and VHCDR3-dependent idiotypic network regulation (Lange et al, 1996, Eur J Immunol 26: 2234-42).

For a further investigation of this subject, we started to analyze the anti-phOx response in mutant mice which lack most of the 13 D gene segments of BALB/c mice and express only one of them. Three different strains are available: Strain Δ D-DFL contains the normal DFL-D-segment *DFL16.1* which preferentially codes for neutral amino acids. Strain Δ D-iD contains a D gene segment with an inverted reading frame (*inverted D* = iD) coding preferentially for charged amino acids. Strain Δ D-rf2 contains one D gene segment in the second reading frame (*reading frame* 2 = rf2) which preferentially codes for hydrophobic amino acids. This project is done in collaboration with Dr. Michael Zemlin (Pediatric Clinics, University of Marburg, Germany) who participated in the generation of these mice in the laboratory of Prof. Dr. Harry Schroeder (Birmingham, USA).

The hitherto obtained results in strains Δ D-iD and Δ D-rf2 show already a) that the full repertoire of D gene segments is essential for a good immune response and b) that the normally dominant Ox1 idio type is only of minor importance in these mice although the typical VHCDR3 sequence DRG can principally, but rarely be generated. Further investigations will show how the immune maturation develops in these mice (Ahmad Trad, Doctoral Thesis, Kiel 2009).

D Publications 2008/2009

Publications 2008	Impact Factor
1. Lange H, Zemlin M, Tanasa RI, Trad A, Weiss T, Menning H, Lemke H (2008) Thymus-independent type 2 antigen induces a long-term IgG-related network memory. Mol. Immunol. 45 : 2847-2860.	3.555
Publications 2009	Impact Factor
1. Tanasa RI, Trad A, Lange H, Grötzinger J, Lemke H (2009) Allergen IgE-isotype-specific suppression by maternally derived monoclonal anti-IgG-idiotype. Allergy. (in press)	6.204
2. Lemke H, Tanasa RI, Trad A, Lange H (2009) Benefits and burden of the maternally-mediated immunological imprinting. Autoimmun. Rev. 8 : 394-399.	5.371

Impact factors 2008: 3.555

Impact factors 2007: 11.575

Total impact factors 2008/2009: 15.13

E Grants

Currently no grants

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Prof Dr. rer. nat. Joachim Grötzinger



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Laura Ruschkies

Technicians:

Britta Hansen

Ursula Mundt

Jessica Schneider (part-time)

Janina Schröder (Trainee Lab. Technician)

C. Research Report

C.1. Cytokines and their receptors

Interleukin-27 is a heterodimeric protein, which consists of the two proteins p28 and EBI-3. Whereas p28 is thought to belong to the four-helix bundle family of cytokines, EBI-3 is a soluble cytokine receptor containing the typical signatures of this family. The signaltransducers of IL-27 are gp130 and WSX-1, which are activated by IL-27 upon binding to their extracellular parts. EBI-3 as well as WSX-1 contains a so called cytokine binding region, which consists of two domains with the signature of two conserved cysteine bridges in the N-terminal and a conserved WSXWS motive in the C-terminal domain. Like interleukin-6 and interleukin-15, p28 is able to interact with three different receptor chains. The structure, the location and the affinity of the different receptor-binding epitopes of p28 to their respective receptor chains are not known. The goal of the planned work is to establish a structure/function relationship for p28 and its receptors EBI-3 and WSX-1. Therefore, we will solve the structure of p28 and the cytokine binding regions of EBI-3 and WSX-1 by multidimensional heteronuclear NMR spectroscopy. By using one ^{15}N labeled component and a second unlabeled component we will identify the amino-acid residues involved in the interaction between the two molecules by ^{15}N - ^1H correlated NMR spectra. The identification of the residues crucial for the ligand/receptor interaction and the three-dimensional structure

of these molecules is the prerequisite for the development of therapeutically interesting antagonists and/or agonists.

C.2. Antimicrobial proteins

The primary defence of an organism against pathogens is performed by the physical barrier of the epidermis and the epithel. The secondary defence is mediated by the epidermal cells, by their secretion of antimicrobial peptides and proteins, like defensins and pore forming proteins. The molecular mechanisms of their interaction with the pathogens are not well understood. The enlightening of the three dimensional structure of these proteins is the basis for understanding their mechanisms of action. In this project the three-dimensional structures of the newly discovered antimicrobial peptides of *Caenorhabditis elegans*, *Ciona intestinalis* and *Hydra* were solved by NMR spectroscopy.

D. Publications 2008/2009

Publications 2009	Impact Factor
1. Xun Y, Tremouilhac P, Carraher C, Gelhaus C, Ozawa K, Dixon N.E, Leippe M, Grötzinger J, Dingley AJ, Kralicek AV. (2009) Cell-free synthesis and combinatorial selective ¹⁵ N labeling of the cytotoxic protein amoebapore A from <i>Entamoeba histolytica</i> . Prot Express and Purification 68, 22-27.	1.621
2. Dhir V, Reisch N, Bleicken CM, Lebl J., Kamrath C, Schwarz HP, Grötzinger J, Sippell WG, Riepe FG, Arlt W, Krone N. (2009) Steroid 17-Hydroxylase Deficiency: Functional Characterization of Four Mutations (A174E, V178D, R440C, L465P) in the <i>CYP17A1</i> Gene. J Clin Endocrinol Metab 94, 3058-3064.	6.325
3. Lange W, Geißendörfer J, Schenzer A, Grötzinger J, Seebohm G, Friedric T, Schwake M. (2009) Refinement of the binding site and mode of action of the anticonvulsant retigabine on KCNQ K ⁺ channels. Mol Pharmacol 75, 1-9.	4.711
4. Bosch TCG, Augustin R, Anton-Erxleben F, Fraune S, Hemmrich G, Zill H, Rosenstiel P, Jacobs G, Schreiber S, Leippe M, Stanisak M, Grötzinger J, Jung S, Podschun R, Bartels J, Harder J, Schröder JM. (2009) Uncovering the evolutionary history of innate immunity: the simple metazoan hydra uses epithelial cells for host defence. Dev Comp Immun 33, 559-569.	2.833
5. Andrä J, Hammer MU, Grötzinger J, Jakovkin I, Lindner B, Vollmer E, Fedders H, Leippe M, Gutschmann T. (2009) Significance of loop structure and arginine residues for the antibacterial activity of arenicin-1 and its interaction with phospholipid and lipopolysaccharide model membranes. Biol Chem 390, 337-340.	3.035
6. Bruhn O, Cauchard J, Gelhaus C, Thaller G, Leippe M, Grötzinger J. (2009) Antimicrobial properties of the equine α -defensin DEFA1 against pathogenic bacteria of the horse. Vet Immunol Immunop 130, 102-110.	1.907
7. Jung S, Dingley AJ, Augustin R, Anton-Erxleben F, Stanisak M, Gelhaus C, Gutschmann T, Hammer MU, Podschun R, Leippe M, Bosch TCG, Grötzinger J. (2009) Hydramacin-1: Structure and antibacterial activity of a peptide from the basal metazoan Hydra. J Biol Chem 284, 1896-1905.	5.520
8. Michalek M, Gelhaus C, Hecht O, Podschun R, Schröder JM, Leippe M, Grötzinger J. (2009) The human antimicrobial protein Psoriasin acts by permeabilization of bacterial membranes. Dev Comp Immun 33, 740-746.	2.833
9. Adam N, Rabe B, Suthaus J, Grötzinger J, Rose-John S, Scheller J. (2009) Understanding receptor independent gp130 activation by viral interleukin-6. J Virol 83, 5117-5126.	5.308
10. Krone N, Grötzinger J, Holterhus PM, SippellWG, Schwarz HP, Riepe FG. (2009) Congenital adrenal hyperplasia due to 11-hydroxylase deficiency – insights from two novel CYP11B1 mutations (p.M92X, p.R453Q) . Horm Res 72, 281-286.	2.285
11. Tanasa RI, Trad A, Lange H, Grötzinger J, Lemke H. (2009) Allergen IgE-isotype-specific suppression by maternally derived monoclonal anti-IgG-	6.204

idiotype. Allergy (in press).	
12. Regenhard R, Leippe M, Schubert S, Podschun R, Kalm E, Grötzinger J, Looft C. (2009) Antimicrobial Activity of Bovine Psoriasin. Veterinary Microbiology 136, 335-340.	2.370
13. Bleicken C, Loidi L, Dhir V, Parajes S, Quinteiro C, Dominguez F, Grötzinger J, Sippell WG, Riepe FG, Arlt W, Krone N. (2009) Functional characterization of three CYP21A2 sequence variants employing a yeast co-expression system. Human Mutation 30, 443-450.	7.033

Publications 2008	Impact Factor
1. Tenhumberg S, Waetzig GH, Chalaris A, Rabe B, Seegert D, Scheller J, Rose-John S, Grötzinger J. (2008) Structure guided optimization of the interleukin-6 transsignalling antagonist sgp130. J Biol Chem 283, 27200–27207.	5.520
2. Riepe FG, Hiort O, Grötzinger J, Sippell WG, Krone N, Holterhus PM. (2008) Functional and structural consequences of a novel point mutation in the <i>CYP21A2</i> gene causing Congenital Adrenal Hyperplasia: Potential relevance of helix C for POR-CYP21 interaction. J Clin Endocrinol Metab. 93, 2891-2896.	6.325
3. Dingley AJ, Lorenzen I, Grötzinger J. (2008) NMR analysis of viral protein structures. Methods Mol Biol. 451:441-62.	
4. Bräsen C, Schmidt M, Grötzinger J, Schönheit P. (2008) Reaction Mechanism and structural model of ADP-forming acetyl-CoA synthase from the hyperthermophilic archaeon pyrococcus furiosus: Evidence for a second active site histidine residue. J Biol Chem 283, 15409-15418.	5.520
5. Fedders H, Michalek M, Grötzinger J, Leippe M. (2008) An exceptional salt tolerant antimicrobial peptide derived from a novel gene family of hemocytes of the marine invertebrate <i>Ciona intestinalis</i> . Biochem. J. 416, 65-75.	4.371
6. Welzel M, Wüstemann N, Simic-Schleicher G, Dörr HG, Schulze E, Shaikh G, Clayton P, Grötzinger J, Holterhus PM, Riepe FG. (2008) Carboxyterminal mutations in 3 β -hydroxysteroid dehydrogenase cause congenital adrenal hyperplasia due to 3 β -hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab. 93, 1418-1425.	6.325
7. Andrä J, Jakovkin I, Grötzinger J, Hecht O, Krasnosdembskaya AD, Goldmann T, Gutschmann T, Leippe M. (2008) Structure and mode of action of the antimicrobial peptide arenicin. Biochem J 410, 113-122.	4.371

Impact factors 2009: 51.985

Impact factors 2008: 32.432

Total impact factors 2008/2009: 84.417

E. Grants

- E.1. Strukturaufklärung antimikrobieller Peptide und Proteine mit Hilfe der NMR-Spektroskopie
DFG SFB 617-A9, Fördersumme (2005 – 2009) 274,800 €
- E.2. Rekombinante Synthese humaner antimikrobieller Peptide und Proteine
DFG SFB 617-Z2, Fördersumme (2005 – 2009) 238,800 €
- E.3. Strukturaufklärung des Interleukin-15 / Interleukin-15-Rezeptor Komplexes und extrazellulärer gp130 Domänen
DFG SFB 415-B7, Fördersumme (2007 – 2010) 222,400 €
- E.4. Ursprüngliche cytolytische und antimikrobielle Mechanismen von humanpathogenen und freilebenden Protozoen im Vergleich zu höheren Organismen
DFG Einzelantrag Leippe/Grötzinger: Fördersumme (2007 – 2008) 82,000 €

- E.5. Exzellenzcluster Inflammation at Interfaces, Integrated Research Network G: NOD-like receptors, TP5: NOD2 structure, Fördersumme (2008-209); 170.000 €;
- E.6. Exzellenzcluster Inflammation at Interfaces, Integrated Research Network F: gp130 signalling, TP1: Structural and molecular desing of novel cytokine antagonists, Fördersumme (2008-209); 200.000 €;

4. Research Group Prof. Dr. Jürgen Scheller

A Group Leader:

Prof. Dr. Jürgen Scheller



B Lab Members:

Post-Doc:

Dr. Doreen Floss (since 2009)

Doctoral Students:

Christin Dewitz

Christoph Garbers

Katja Müller

Nina Adam

Olga Braun

Ulrike May (until 2009)

Medical doctoral students

Anne Oberdörster

Ingo Plagmann (2007-2008)

Wolfgang Thaiss

Diploma students

Anna Tillmann (2008)

Timo Effenberger (2009)

Jan Sommer (2009/2010)

Technicians:

Stefanie Schnell (until 2009)

Annett Lickert (since 2009)

Jessica Schneider (part-time)

Thies Reick (Trainee Lab. Technician)

C Research Report

C.1. Role of Interleukin 6 *in vitro* and *in vivo*

The immunoregulatory cytokine Interleukin-6 (IL6) acts in a pro- and anti-inflammatory fashion. Synthesized by myeloid cells, fibroblasts and endothelial cells, IL6 on target cells, binds to the IL6 receptor (IL6R) and signals via complex formation with the ubiquitously expressed gp130 receptor. Paradoxically, most cells, which respond to IL6 during inflammatory states do not express the IL6R and are themselves not responsive to the cytokine. A naturally occurring soluble form of the IL6R renders all cells responsive to IL6. Interleukin 6 (IL6) trans-signaling has emerged as a prominent regulator of immune responses during both innate and acquired immunity. Regulation of IL6 trans-signaling is reliant upon the release of soluble IL6 receptor (sIL6R), which binds IL6 to

create an agonistic IL6/sIL6R complex capable of activating cell types that would not normally respond to IL6 itself.

Here we developed a transgenic strategy based on the overexpression of the soluble form of gp130, which specifically blocks all IL6 responses mediated by the soluble IL6R but does not affect IL6 responses via the membrane-bound IL6R. In these mice, inflammatory processes are blocked as in IL6^{-/-} mice, strongly arguing for a major role of the soluble IL6R during inflammation *in vivo*.

Colitis-associated cancer (CAC) is the most serious complication of inflammatory bowel disease. Proinflammatory cytokines have been suggested to regulate preneoplastic growth during CAC tumorigenesis. Interleukin 6 (IL-6) is a multifunctional NF- κ B-regulated cytokine that acts on epithelial and immune cells. Using genetic tools, we now demonstrate that IL-6 is a critical tumor promoter during early CAC tumorigenesis. In addition to enhancing proliferation of tumor-initiating cells, IL-6 produced by lamina propria myeloid cells protects normal and premalignant intestinal epithelial cells (IECs) from apoptosis. The proliferative and survival effects of IL-6 are largely mediated by the transcription factor Stat3, whose IEC-specific ablation has profound impact on CAC tumorigenesis. Thus, the NF- κ B-IL-6-Stat3 cascade is an important regulator of the proliferation and survival of tumor-initiating IECs.

Human herpesvirus 8 encodes a viral version of interleukin-6 (vIL-6) which shows 25% sequence homology with human IL-6. In contrast to human IL-6, which first binds to the IL-6 receptor (IL-6R) and only subsequently associates with the signal transducing receptor subunit gp130, vIL-6 has been shown to directly bind to gp130 without the need of IL-6R. As a functional consequence, vIL-6 can activate far more target cells in the body since all cells express gp130, but only cells such as hepatocytes and some leukocytes express IL-6R. We sought to understand which amino acid sequences within the vIL-6 protein were responsible for its ability to bind and activate gp130 independent of IL-6R. As a first approach, we constructed chimeric IL-6 proteins in which all known gp130 interacting sites (sites II and III) were sequentially transferred from vIL-6 into the human IL-6 protein. To our surprise, human IL-6 carrying all gp130 interacting sites from vIL-6 did not show IL-6R-independent gp130 activation. Even more surprisingly, the loop between helix B and C of vIL-6, clearly shown in the crystal structure not to be in contact with gp130, is indispensable for direct binding to and activation of gp130. This points to an IL-6R induced change of site III conformation in human IL-6, which is already preformed in vIL-6. These data indicate a novel activation mechanism of human IL-6 by the IL-6R that will be important for the construction of novel hyperactive cytokine variants.

C.2. Single domain antibodies

Tumor necrosis factor (TNF) plays an important role in chronic inflammatory disorders, such as Rheumatoid Arthritis and Crohn's disease. Recently, monoclonal Camelidae variable heavy-chain domain-only antibodies (VHH) were developed to antagonize the action of human TNF (hTNF). Here, we show that hTNF-VHH does not interfere with hTNF trimerization, but competes with hTNF for hTNF-receptor binding. Moreover, we describe posttranslational dimerization and multimerization of hTNF-VHH molecules *in vitro* catalyzed by microbial transglutaminases (MTG). The ribonuclease S-tag-peptidase was shown to act as a peptidyl substrate in covalent protein cross-linking reactions catalyzed by MTG from *Streptomyces mobaraensis*. The S-tag sequence was C-terminally fused to the hTNF-VHH and the fusion protein was expressed and purified from *Escherichia coli* culture supernatants. hTNF-VHH-S-tag fusion proteins were efficiently dimerized and multimerized by MTG whereas hTNF-VHH was not susceptible to protein crosslinking. Cell cytotoxicity assays, using hTNF as apoptosis inducing cytokine, revealed that dimerized and multimerized hTNF-VHH proteins were much more active than the monomeric hTNF-VHH. We hypothesize that improved inhibition by dimeric and multimeric single chain hTNF-VHH proteins is caused by avidity effects.

C.3. Elastin-like polypeptides

Elastin-like polypeptides (ELPs) are highly biocompatible and exhibit a potentially highly useful property – that of a thermally responsive reversible phase transition. These characteristics make ELPs attractive for drug delivery, appealing as materials for tissue repair or engineering, and improve the efficiency with which recombinant proteins can be purified. ELP fusion proteins (referred to as 'ELPylation') inherit the reversible phase transition property. ELPylation technology has recently been extended to plant cells, and a number of plant-based expression systems have been evaluated for the production of ELPylated proteins. Here, we discuss recent developments in ELP technology and the substantial potential of ELPs for the deployment of transgenic plants as bioreactors to synthesize both biopharmaceuticals and industrial proteins.

D Publications 2008/2009

Publications 2008	Impact Factor
1. West, M.A., Prescott, A.R., Chan, K.M., Zhou, Z., Rose-John, S., Scheller, J. and C. Watts (2008). TLR-ligand induced podosome disassembly in dendritic cells is ADAM17 dependent. <i>J. Cell. Biol.</i> 182:993-1005.	9.598
2. Tenhumberg, S., Waetzing, G.H., Chalaris, A., Rabe, B., Seegert, D., Scheller, J., Rose-John, S., and J. Grötzinger (2008). Structure guided optimization of the Interleukin-6 Transsignaling antagonist sgp130. <i>J. Biol. Chem.</i> 283: 27200-27207.	5.581
3. Sudarman, E., Bollati-Fogolin, M., Hafner, M., Müller, W., Scheller, J., Rose-John, S. and J. Eichler (2008). Synthetic Mimetics of the gp130 binding site for viral Interleukin 6 as inhibitors of the vIL6-gp130 interaction. <i>Chem. Biol. Drug Des.</i> 71: 494-500.	0,409
4. Marino, M., Scuderi, F., Provenzano, C., Scheller, J., Rose-John, S. and E. Bartoccioni (2008). IL-6 regulates MCP-1, ICAM and IL6-expression in human myoblasts. <i>J. Neuroimmunol.</i> 196:41-8.	2.880
5. Floss, D.M., Sack, M., Stadlmann, J., Rademacher, T., Scheller, J., Stöger, E., Fischer, R. and U. Conrad (2008). Biochemical and functional characterization of anti-HIV antibody ELP fusion proteins from transgenic plants. <i>Plant Biotechnol. J.</i> 6: 379-91.	4.419
6. Rabe, B., May, U., Chalaris, A., Seegert, D., Waetzig, G., Rose-John, S. and J. Scheller (2008) Interleukin 6 receptor goes soluble: Abrogated inflammation by transgenic blockade of sIL6R-responses. <i>Blood</i> 111: 1021-1028.	10.370
7. Benrick, A., Pernilla, J., Wernstedt, I., Gustafsson, M., Scheller, J., Eriksson, A. L., Borén, J., Hedner, T., Ohlsson, C., Härd, T., Rose-John, S., J. O. Jansson (2008) A non-conservative polymorphism in the IL-6 signal transducer (IL6ST)/gp130 is associated with myocardial infarction in a hypertensive population. <i>Regulatory Peptides</i> 146:189-96.	2.442
Publikationen 2009	Impact Factor
1. Nowell, M.A., Williams, A.S., Carty, S.A., Scheller, J., Hayes, A.J., Jones, G.W., Richards, P.J., Slinn, S., Ernst, M., Jenkins, B.J., Topley, N., Rose-John, S. and S.A. Jones (2009). Therapeutic targeting of IL-6 trans-signaling counteracts STAT3 control of the inflammatory infiltrate in experimental arthritis. <i>J. Immunol.</i> 182:613-22.	6.068
2. Grivennikov, S., Karin, E., Teric, J., Mucida, D., Yu, G.Y., Vallabhapurapu, S., Scheller, J., Rose-John, S., Cheroutre, H., Eckmann, L. and M. Karin (2009). IL-6 and STAT3 are required for survival of intestinal epithelial cells and development of colitis associated cancer. <i>Cancer Cell.</i> 15(2):103-13.	22.321
3. Baier, P.C., May, U., Scheller, J., Rose-John, S. and T. Schifflholz (2008). Impaired hippocampus-dependent and independent learning in IL-6 deficient mice. <i>Behav. Brain. Res.</i> 613(1-3):141-145.	2.855
4. Prakash, R., Satory, D., Dray, E., Papusha, A., Scheller, J., Kramer, W., Krejci, L., Klein, H., Haber, J.E., Sung, P. and G. Ira (2009) Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. <i>Genes Dev.</i> 23, 67-79.	15.487

5.	Adam, N., Rabe, B., Suthaus, J., Grötzinger, J., Rose-John, S. and J. Scheller (2009). Unraveling Viral Interleukin 6 binding to gp130 and activation of STAT-Signaling Pathways independent of Interleukin 6-Receptor. <i>J. Virol.</i> 83:5117-26.	5.332
6.	Plagmann, I., Chalaris, A., Nedospasov, S., Posenstiel, P., Rose-John, S. and J. Scheller (2009) Transglutaminase-catalyzed covalent multimerization of camelidae anti-human TNF α single domain antibodies improves neutralizing capacity of human TNF α . <i>J. Biotechnol.</i> 142: 170-8.	3,015
7.	May, U., Schiffelholz, T., Baier, P.C., Krueger, J.M., Rose-John, S. and J. Scheller (2009). IL6-trans-signalling increases rapid-eye-movement sleep in rats. <i>EJP.</i> 613: 141-5.	2.565
8.	Benedict, C, Scheller, J., Rose-John, S., Born, J. and L. Marshall (2009). Enhancing influence of intranasal interleukin-6 on slow wave activity and memory consolidation during sleep. <i>FASEB J.</i> 23:3629-36.	7.049
9.	Floss, D., Sack, M., Acalis, E., Stadlmann, J., Quendler, H., Rademacher, T., Stoger, E., Scheller, J., Fischer, R. and U. Conrad (2009) Influence of Elastin-like Peptide Fusion on Quantity and Quality of a Tobacco-produced Anti HIV Antibody. <i>Plant Biotechnol. J.</i> 7:899-913.	4.419
10.	Sina, C., Förster, M., Derer, S., Hildebrand, F., Chalaris, A., Rabe, B., Scheller, J., Gavrilowa, O., Rehmann, A., Ott, S., Häslner, R., Li, J., Nikolaus, S., Fölsch, U.R., Rose-John, S., Schreiber, S. and P. Rosenstiel (2009) G-protein coupled receptor 43 (GPR43) is essential for neutrophil recruitment during intestinal inflammation. <i>J. Immunol.</i> 183:7514-22.	6.00
11.	Rose-John, J., Mitsuyama, K., Matsumoto, S., Thaiss, W.M. and J. Scheller (2009) Interleukin-6 Trans-Signaling and Colonic Cancer Associated with Inflammatory Bowel Disease. <i>Current Pharmacological Design</i> , 15: 2095-103. Review.	4.868

Impact factors 2008: 35.29

Impact factors 2009: 70.967

Total impact factors 2008/2009: 106.257

E Grants

- E.1. Jürgen Scheller and Stefan Rose-John
Der lösliche Interleukin-6-Rezeptor: Entstehung und physiologische Bedeutungen
Sonderforschungsbereich 415, Teilprojekt B5, Fördersumme (2007 – 2010) 248.700 €
- E.2. Stefan Rose-John, Jürgen Scheller, Jan Born, Dunja Hinze-Selch
The function of the gp130-signaling-family for sleep and plasticity
Sonderforschungsbereich 654, Teilprojekt C5, Fördersumme (2005 – 2009) 444.600 €
- E.3. Stefan Rose-John and Jürgen Scheller
The function of the gp130-signaling-family for sleep and plasticity
Sonderforschungsbereich 654, Teilprojekt C5, Fördersumme (2009 – 2013) 387.200 €
- E.4. Jürgen Scheller and Werner Solbach
Influences of sleep, hormones and cytokines on circadian rhythm of T cell activity
Sonderforschungsbereich 654, Teilprojekt C8, Fördersumme (2009 – 2013) 353.600 €
- E.5. Exzellenzcluster Inflammation at Interfaces, Integrated Research Network F: gp130 signalling, TP3: Cytokine Signalling, Fördersumme (2009); 132.600 €; Miniproposal (2009): 50.000 €

5. Research Group Prof. Dr. Paul Saftig

A Lab Leader:

Prof. Dr. Paul Saftig



B Lab Members:

Group leaders:

Michael Schwake
Bernd Schröder
Judith Blanz

Post-Docs:

Dr. rer. nat. Andrea Rittger
Dr. rer. nat. Jenny Schröder

Doctoral Students:

Marion Willenborg
Alexander Schneede
Silvio Weber
Johannes Prox
Telly Savallas
Christina Wehling
Johann Groth
Christina Zachos
Michelle Danahar
Janna Schneppenheim

Medical Students:

Meike Lüdemann
Miriam Wagner

Diploma Students:

Daniel Mielkerei
Mia Schmied
Katharina Rothe
Jörg Behmke
Nur Güneli
Judith Pohanke
Christian Raab

Bachelor:
Friederike Zunke

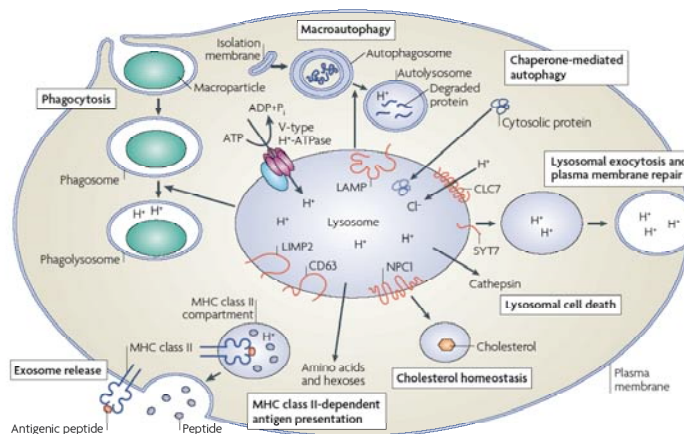
Technician:
Marlies Rusch
Katharina Stiebeling
Maike Langer
Inez Götting
Sebastian Held

Trainee Lab. Technician
Tobias Lehmann
Steffie Jessen
Merjem Senkara
Raphael Kurz
Rouven Bahn
Marvin Murowski

Secretary
Gundula Hohn

C Research Report

C.1. Biology of lysosomes:



Major functions of lysosomal membrane proteins. The lysosome is a central, acidic organelle that is involved in the degradation of macromolecules through the activity of lysosomal hydrolases. Lysosomes are crucial for the maturation of phagosomes to phagolysosomes in phagocytosis, which is important for cellular pathogen defence. The macroautophagy pathway mediates the turnover of cytoplasmic components, such as organelles and large complexes, and is involved in cell death and proliferation. Macroautophagy depends on the fusion of lysosomes with autophagosomes to create autolysosomes, in which degradation occurs. Macroautophagy and chaperone-mediated autophagy, a direct lysosomal transport process for cytosolic proteins, are regulated by lysosome-associated membrane proteins (LAMPs). Lysosomal exocytosis and plasma membrane repair are Ca^{2+} and synaptotagmin 7 (SYT7)-dependent fusion events, which are possibly involved in pathogen entry, autoimmunity and neurite outgrowth. The lysosomal cell death pathway is triggered by a release of lysosomal cathepsins through an unknown mechanism. Cellular cholesterol homeostasis is controlled by lysosomal cholesterol efflux through Niemann–Pick C1 protein (NPC1). Major histocompatibility complex (MHC) class II-dependent antigen presentation requires lysosomal proteases and membrane proteins. The release of exosomes is thought to be involved in adaptive immune responses. Lysosomal membrane proteins are also involved in the transport of newly synthesized hydrolases to the lysosome (for example, lysosomal integral membrane protein 2 (LIMP2)) and across the lysosomal membrane (for example, the V-type H^{+} -ATPase complex and chloride channel protein 7 (CLC7)). (from Saftig & Klumpermann; *Nature Reviews in Cell and Molecular Biology*, 2009, 10:623-635)

Lysosomes, lysosomal membrane proteins, hydrolases and lysosomal storage disease:

Lysosomes are ubiquitous organelles that constitute the primary degradative compartments of the cell. They receive their substrates through endocytosis, phagocytosis or autophagy. The catabolic function of lysosomes is complemented by lysosome-related organelles (LROs), such as melanosomes, lytic granules, major histocompatibility complex (MHC) class II compartments and platelet dense granules. LROs share many properties with lysosomes, but they also contain celltype specific proteins and might require additional cellular machinery for their biogenesis. Lysosomes and LROs are involved in various physiological processes, such as cholesterol homeostasis, plasma membrane repair, bone and tissue remodelling, pathogen defence, cell death and cell signalling. These complex functions make the lysosome a central and dynamic organelle and not simply the dead end of the endocytic pathway. Two classes of proteins are essential for the function of lysosomes: soluble lysosomal hydrolases (also referred to as acid hydrolases) and integral lysosomal membrane proteins (LMPs). Each of the 50 known lysosomal hydrolases targets specific substrates for degradation, and their collective action is responsible for the total catabolic capacity of the lysosome. In addition to bulk degradation and proprotein processing, lysosomal hydrolases are involved in antigen processing, degradation of the extracellular matrix and initiation of apoptosis. The mammalian lysosome contains ~25 LMPs, but additional LMPs are being revealed. LMPs reside mainly in the lysosomal limiting membrane and have diverse functions, including acidification of the lysosomal lumen, protein import from the cytosol, membrane fusion and transport of degradation products to the cytoplasm. The most abundant LMPs are lysosome associated membrane protein (LAMP1), LAMP2, lysosome integral membrane protein 2 (LIMP2; also known as SCARB2) and the tetraspanin CD63.

Lysosome biogenesis requires integration of the endocytic and biosynthetic pathways of the cell. Lysosomal targeting of newly synthesized lysosomal proteins can be direct, from the *trans*-Golgi network (TGN) to the endosomal system, or indirect, involving transport to the plasma membrane and subsequent endocytosis. The best understood direct pathway is the mannose 6 phosphate receptor (M6PR) mediated transport of lysosomal hydrolases. By contrast, remarkably little is known about the structural and molecular machinery for the transport of LMPs to lysosomes. The significance of tightly regulated LMP trafficking is illustrated by recent findings that describe new and unexpected roles for LMPs in cellular physiology. It is becoming apparent that LMPs can impose specific functions onto the organelles through which they are transported or in which they reside, such as the endoplasmic reticulum (ER), lysosomes and the plasma membrane. Their importance is further highlighted by the discovery of an increasing number of gene mutations that lead to lysosomal dysfunction and disease. In addition, various knockout mice and non mammalian model organisms have highlighted the role of LMPs in cell physiology. We are continuing our efforts to characterize the molecular functions of these proteins for health and disease.

C.2. Towards a new therapy for the lysosomal storage disorder alpha-Mannosidosis:



The European HUE-MAN network is coordinated through activities in our lab. Basic scientists and clinicians contribute to the development of an enzyme replacement therapy for the human alpha-Mannosidosis disease.

Alpha-Mannosidosis is a rare inborn disorder caused by the lack of the lysosomal enzyme α -Mannosidase, resulting in mental retardation, skeletal changes, hearing loss and recurrent infections. The children are often born apparently normal, and their conditions worsen progressively, without any possibility to prevent this evolution. In the children that are born healthy, a therapy initiated at an early age could contribute to a normal development. Today, the most promising therapy for lysosomal storage disorders is enzyme replacement therapy (ERT); where the enzyme lacking in the patient is introduced into the blood stream, from where it is internalised

by the cells and reaches the lysosomes, acting as the endogeneous enzyme. ERT products are on the market today for diseases such as Gaucher and Fabry and clinical trials are underway for a number of other diseases. A correction of storage in many tissues including brain was found after administration of lysosomal acid α -Mannosidase (LAMAN) from bovine kidney, and human and mouse recombinant LAMAN. The present project (PI: Judith Blanz) is of great value for the introduction of recombinant human lysosomal alpha-mannosidase into the first clinical trials of ERT in patients. In line with our plans we have been able to evaluate the benefit of alpha mannosidase ERT in the mouse model and we have made significant advances to get a suitable protocol for treatment of this lysosomal storage disorder. Importantly, the correction of storage has been followed over time in visceral organs but also in the central nervous system. A possible improvement of the brain associated symptoms has been analysed by sophisticated behavioral monitoring of treated and non treated mice, respectively. We have initiated a study in the mouse model of the hitherto unrecognized role of epigenetic factors contributing to the manifestation of the disease. On the other hand a systematic clinical and diagnostic natural history study of the disease in human patients was successfully started. This study enables us to define the critical parameters and hallmarks of the disease which will eventually be used in phase I and phase II clinical trials to evaluate the efficacy of the ERT in patients. Apart from providing sufficient enzyme for the preclinical studies the optimisation of the production of the human recombinant LAMAN enzyme has been pushed forward and will be of major importance for the successful introduction of this enzyme into clinical phase trials. We could successfully develop the enzyme production and the replacement therapy in the mouse model. We have obtained important information about dose and interval of ERT treatment, about the mode of correction in peripheral neurons and neurons of the CNS, about behavioral profiles of the mouse model with and without ERT, about differentially expressed genes in treated and untreated mice. We could further analyse in detail the oligosaccharide storage by newly developed mass spectrometry analysis and started to establish this methodology for the analysis for clinical use.

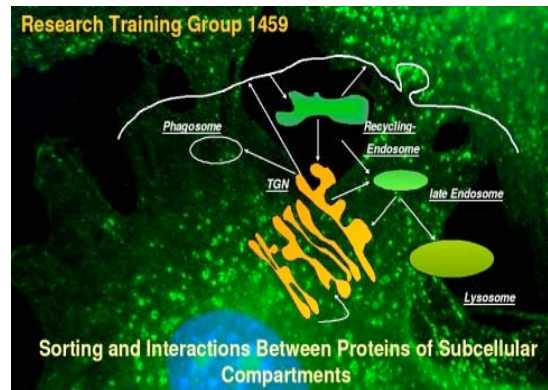
C.3. Biology of the newly identified lysosomal sorting receptor LIMP-2:

We (PI: Michael Schwake) have identified an unexpected role for the lysosomal membrane protein LIMP-2 as a sorting receptor required for the delivery of beta-glucocerebrosidase to lysosomes. The Mannose-6-Phosphate-receptor pathway has been very well characterized as a major route for the sorting of lysosomal enzymes; however, the mechanism for the intracellular targeting of beta-glucocerebrosidase to lysosomes has been unclear until now. Our findings that beta-glucocerebrosidase associates with LIMP-2, that these proteins colocalize in intracellular vesicular compartments, and that the activity, levels, and localization of beta-glucocerebrosidase exhibit a dramatic correlation with the presence or absence of LIMP-2 reveal that beta-glucocerebrosidase independence of Mannose-6-Phosphate-based sorting mechanisms is almost certainly a consequence of its routing via LIMP-2 through the lysosomal membrane protein delivery pathway.

Action myoclonus-renal failure syndrome (AMRF) is caused by mutations in LIMP-2. To date, six AMRF-causing mutations have been described, including splice site, missense and nonsense mutations. All mutations lead to a retention of LIMP-2 in the endoplasmic reticulum (ER) but affect the binding to b-GC differentially. The LIMP-2 segment 145–288, comprising the nonsense mutations, contains a highly conserved coiled-coil domain, which we suggest determines beta-glucocerebrosidase binding. Disruption of the helical arrangement and amphiphatic nature of the coiled-coil domain abolishes beta-glucocerebrosidase binding. In contrast to the reduced binding properties of the nonsense mutations, the only missense mutation (H363N) found in AMRF leads to increased binding of beta-glucocerebrosidase to LIMP-2, indicating that this highly conserved histidine modifies the affinity of LIMP-2 to its ligand. We suggest that disruption of the coiled-coil structure or AMRF disease-causing mutations abolish beta-glucocerebrosidase binding, indicating the importance of an intact coiled-coil structure for the interaction of LIMP-2 and beta-glucocerebrosidase.

C.4. The graduate research training school (GRK1459): Sorting and interaction between proteins of subcellular compartments

The DFG-Research Training Group 1459 is co-coordinated by our group and scientists from the University Medical Center Hamburg-Eppendorf and the Bernhard-Nocht-Institute for Tropical Medicine in Hamburg. The program is open to students with a diploma/master in natural sciences and medical students. The general topic of the Research Training Group is sorting and transport of selected proteins within the Golgi apparatus and endosomal compartments. In these organelles the decision is made whether a newly synthesized protein reaches its target via the secretory/biosynthetic pathway, or a recently internalized molecule (or bacterium) reaches its intracellular destination via the endocytic/phagocytic pathway. Missorted proteins may lead to loss of function in their target organelles, that may affect the well being of the cell and the organism as a whole.



Therefore, the experimental approaches are related to diseases. By focussing on selected model proteins, basic mechanisms of the biogenesis of intracellular compartments as well as the balance of membrane transport between organelles and the interplay between cytosolic and membrane proteins will be investigated. The majority of projects addresses sorting and transport processes under pathological conditions in cells derived from patients or mouse models of human diseases, or cells infected by bacteria or in parasite cells. New insights into the interactions between resident proteins of endosomes and the Golgi apparatus with components of the vesicular transport machinery and the actin cytoskeleton will be expected. A better understanding of cellular responses to endogenous mutant proteins or exogenous pathogens will enable the development of novel therapeutic strategies. Different experimental approaches such as ultrastructural analysis of cellular compartments, genomics, biochemistry, time-resolved imaging, and structural biology will be applied and improve our understanding of spatial and dynamic aspects of membrane transport or translocation. Students will go through a three year curriculum of academic as well as non-academic courses in molecular and cellular biology, biochemistry, infectiology, microbiology, and molecular biomedicine. The Research Training Group offers a continuous educational program with lectures, practical courses, seminars, regular report meetings and an international symposium every two years. The practical courses consist of several three-day hands training units attended by up to 4 students. The company OLYMPUS is associated to the Research Training Group and offers additional seminars on new developments in microscopy and practical courses. Seminars will be given by leading scientists and will foster a broad view on current topics of molecular life sciences. It is expected that each student spends 1-3 months abroad in a laboratory cooperating in the research field. The program provides a broad education, not just on the specific topic of the thesis, but also in research topics of the other participating groups.

C.5. Discovery and elucidation of the functions of new and unknown lysosomal membrane proteins:

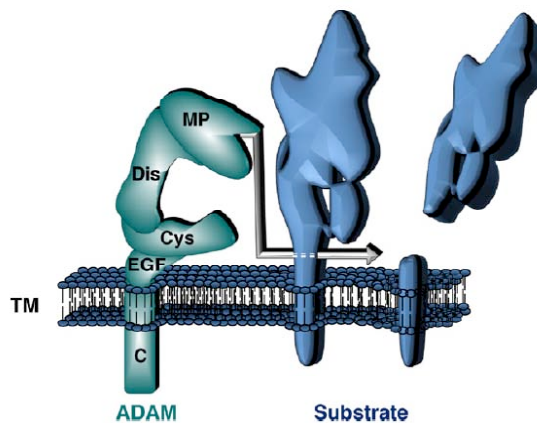
The identification of new lysosomal membrane proteins by subproteomic approaches (PI: Bernd Schröder), in which new members of the lysosomal membrane are being investigated is another focus in the lab. The functional characterization of these new members of the lysosomal membrane will be done using biochemical and mouse genetic approaches. Of special interest in these context are two proteins of hitherto unknown function, DIRC2 and TMEM192.

The concept of regulated intramembrane proteolysis has emerged over the last decades as a novel concept of cellular signalling. One group among the proteases being capable of cleaving substrates within the phospholipid bilayer are the signal-peptide-peptidase (SPP) and its homologues, the signalpeptide-peptidase-like proteins (SPPL2a, -2b, -2c, -3). SPPL2a is present in membranes of lysosomes/late endosomes whereas SPPL2b was reported to reside at the plasma membrane as well as in endosomal compartments. To date, only TNF α , Fas Ligand (FasL) and Bri2(Itm2b) have been identified as substrates of SPPL2a/b. In the case of TNF α it was demonstrated that the proteolytic release of the TNF α intracellular domain influences gene expression and induces the synthesis of the pro-inflammatory cytokine IL12. Two of the known substrates suggest a regulatory function of SPPL2a and SPPL2b in the context of the immune system. In order to analyse functions of SPPL2a and SPPL2b in a complex *in vivo* system, mouse lines deficient in either of the two proteases have been generated. Preliminary immunological phenotyping has revealed major abnormalities in SPPL2a^{-/-} mice.

C.6. Proteolysis in or at membranes

Proteolytic ectodomain release, a process known as "shedding", has emerged as a key mechanism for regulating the function of a diversity of cell surface proteins. Shedding of integral membrane proteins is to our knowledge limited to type I and type II transmembrane proteins or GPI-anchored molecules, in which the cleavage site is generally located close to the membrane surface. A Disintegrin And Metalloproteinases (ADAMs) have emerged as the major proteinase family that mediates ectodomain shedding. ADAM-mediated shedding is important in a number of biological processes such as the interaction of sperm and egg, cell fate determination, cell migration,

wound healing, neurite and axon guidance, heart development, immunity, cell proliferation and angiogenesis. It is estimated that up to 4% of the proteins on the cell surface undergo ectodomain shedding affecting functionally diverse proteins, such as cadherins, L-selectin, Fas ligand, TNF- α , EGFR ligands, ErbB2, ErbB4, Amyloid Precursor Protein (APP), Notch receptor, Notch ligands and many others. ADAMs as proteins of about 750 amino acid length, are characterized by a conserved domain structure, consisting of an N-terminal signal sequence followed by a prodomain, a metalloproteinase domain, a disintegrin domain with a cysteine-rich region, a transmembrane domain and a cytoplasmic tail. ADAM-mediated shedding is both constitutive and inducible, dependent on G-protein coupled receptors, protein kinase C, intracellular Ca^{2+} levels, membrane lipid composition and other experimental and natural stimuli. Also modulation of ADAM activity by removal of the inhibitory prodomain, by changing their intracellular distribution and by interaction of proteins, and/or posttranslational modifications of their cytoplasmic tails play a role in the regulation of ectodomain shedding. Dysregulation of ectodomain shedding is associated with autoimmune and cardiovascular diseases, infection, inflammation and cancer. Therefore, ADAMs are attractive targets for novel therapies. It becomes increasingly clear that further research especially on the regulation and control of ADAM activity, ADAM redundancy in substrate processing, ADAM structure, interaction of ADAM with regulatory proteins and the physiological elucidation of the relevance of ectodomain shedding is needed. Regarding the latter aspect, the use of conditionally targeted mice in conjunction with disease models will be extremely helpful to elucidate the role of these proteins in selected tissues and developmental stages.



Postulated domain structure of ADAMs. ADAMs consist of an extracellular domain with an N-terminal prodomain, a metalloprotease domain (MP), a disintegrin domain (Dis), a cysteine-rich domain (Cys) and an EGF domain. Within the cytosolic domain (C) phosphorylation sites or proline-rich regions with SH3 domains are present. The prodomain is removed during maturation. ADAM-mediated shedding of transmembrane proteins leads to the release of soluble extracellular domains and provides a mechanism for the down-regulation of cell surface proteins but also for extracellular signalling.). (from Reiss & Saftig; Seminars in cell and Developmental Biology, 2009, 20:126-137)

Making use of our conventional knockout mice and cell lines derived from these embryos, we could identify a number of ADAM10-specific substrates. We could show that ADAM10-mediated ectodomain shedding modulates the function of cell adhesion molecules and represents an essential process in the regulation of paracrine, juxtacrine and autocrine signaling. We demonstrated that neuronal cadherin (N-cadherin) is cleaved specifically by ADAM10 in its ectodomain. The ADAM10-induced N-cadherin cleavage resulted in changes in the adhesive behaviour of cells and also in a dramatic redistribution of beta-catenin from the cell surface to the cytoplasmic pool, thereby influencing the expression of b-catenin target genes. Analysis of ADAM10-deficient fibroblasts, inhibitor studies, and RNA interference-mediated down-regulation of ADAM10 demonstrated that ADAM10 is also responsible for the constitutive and regulated shedding of E-cadherin in fibroblasts and keratinocytes. ADAM10-mediated E-cadherin release is also regulated by proinflammatory cytokines thereby modulating keratinocyte cohesion in eczematous dermatitis. Interestingly, gamma-Protocadherins (Pcdh), which are enriched at synapses and involved in synapse formation, specification, and maintenance, are also substrates for ADAM10. Our results demonstrated that ADAM10-mediated Pcdh shedding represents the prerequisite for further processing through g-secretase activity leading to the accumulation of Pcdh fragments in the nucleus. It was also revealed by us that ADAM10 regulates the endothelial permeability and T-cell transmigration by shedding of vascular endothelial cadherin.

We were also able to show that ADAM10 and ADAM17 play an important functional role by regulating L1-dependent neuronal cell adhesion, cell migration, and neurite outgrowth. The emerging critical role of ADAM10 for membrane proteolysis is underlined by the fact that we were able to identify in different collaborative projects a number of further important transmembrane proteins which are susceptible for ADAM10-mediated ectodomain shedding and cell signaling. The CXC-Chemokine-ligand 16, CX3CL1 (fractalkine) (24,25), CD44,

betacellulin, Axl, desmoglein2, receptor tyrosine phosphatase, RAGE, Klotho, CD23 and ephrin are examples for the growing list of ADAM10 specific substrates. Interestingly, Bri2 (Itm2b), as a type II-oriented transmembrane protein which is associated with familial British and Danish dementia, is first shed by ADAM10 and subsequently processed by SPPL2A/SPPL2B. Also the apoptosis-inducing Fas ligand (FasL) is a type II transmembrane protein that is involved in the downregulation of immune reactions by activation-induced cell death (AICD) as well as in T cell-mediated cytotoxicity. Using pharmacological approaches in 293T cells, *in vitro* cleavage assays as well as loss and gain of function studies in MEF cells, we could show that the ADAM10 is critically involved in the shedding of FasL which is supposed to be a prerequisite to further intramembrane cleavage by SPPL2 proteases. Interestingly, we could demonstrate that ADAM10 itself is also subject to regulated intramembrane proteolysis. ADAM9 and -15 were identified as the proteases responsible for releasing the ADAM10 ectodomain, and presenilin (gamma-secretase) as the protease responsible for the release of the ADAM10 intracellular domain (ICD). This domain then translocates to the nucleus and localizes to nuclear speckles, thought to be involved in gene regulation. We concluded that ADAM10 performs a dual role in cells, as a metalloprotease when it is membrane-bound, and as a potential signaling protein once cleaved by ADAM9/15 and the gamma-secretase. In collaborative studies with *Carl Blobel, New York*, we used our ADAM10/ADAM17 chimeric expression constructs to show that for PMA-stimulated TGF-alpha shedding, the intact ectodomain of ADAM17, but not its cytoplasmic and transmembrane domains are required. We could also show that Ca⁺⁺ influx and stimulation of the P2X7R signaling pathway activate ADAM10 as sheddase of many ADAM17 substrates in Adam17^{-/-} fibroblasts and primary B cells.

D Publications in 2008/2009

Publications 2008	Impact Factor
1. Saftig P., Eskelinen EL. (2008) Live longer with LAMP-2. <i>Nat Med.</i> 14:909-910	27.553
2. Eskelinen EL, Saftig P. (2008) Autophagy: a lysosomal degradation pathway with a central role in health and disease. <i>Biochim Biophys Acta.</i> 1793:664-673	5.479
3. Partanen S, Haapanen A, Kielar C, Pontikis C, Alexander N, Inkinen T, Saftig P, Gillingwater TH, Cooper JD, Tyynelä J. (2008) Synaptic changes in the thalamocortical system of cathepsin D deficient mice, a model of human congenital neuronal ceroid-lipofuscinosis. <i>J Neuropathol Exp Neurol.</i> 67, 16-29.	5.140
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Publikationen 2009	Impact Factor
1. Reiss K, Saftig P. (2009) The "a disintegrin and metalloprotease" (ADAM) family of sheddases: physiological and cellular functions. <i>Semin Cell Dev Biol.</i> 20:126-137	4.528
2. Saftig P, Klumperman J. (2009) Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. <i>Nat Rev Mol Cell Biol.</i> 10:623-635	35.423
3. Annaert WG, Saftig P. (2009) Regulated intramembrane proteolysis--a story about sheddases and I-CliPs. <i>Semin Cell Dev Biol.</i> 20:125	4.528
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Impact factors 2008: 136.265

Impact factors 2009: 115.705

Total impact factors 2008/2009: 251.97

E GRANTS

- E.1. An integrated approach towards understanding the pathogenesis of CNS and PNS neurodegenerative disorders
IAP Network Project P6, Fördersumme (2007 – 2011) 68.000,00 €
- E.2. Die in vivo Bedeutung lysosomaler Membranproteine bei der intrazellulären Verwertung von Parasiten und Bakterien nach Infektion
DFG SA 683/6-3, Fördersumme (2009 – 2009) 97.000,00 €
- E.3. Die Funktion von ADAM Metallproteasen
Sonderforschungsbereich 415, Teilprojekt B9, Fördersumme (2007 – 2010) 377.400,00 €
- E.4. Towards the development of an effective enzyme replacement therapy for human alpha-mannosidosis HUE-MAN LSHM-CT-2006-018692, Fördersumme (2006 – 2009) 601.240,00 €
- E.5. Exzellenzcluster Inflammation at Interfaces, Integrated research network: NOD-like receptors, TP3: Lysosomes and NLR, Fördersumme (2009-2012); 349.900 €; Miniproposal Fördersumme (2008) 50.000 €
- E.6. Sortierung und Wechselwirkung zwischen Proteinen subzellulärer Kompartimente; Graduiertenkolleg GRK 1459/1 (DFG); Fördersumme (2008-2012) 374.855 €
- E.7. Design of zinc metalloenzyme targeted drugs using an integrated technology approach (DeZnit) Fördersumme (2007-2010) 150.000 €

6. Research Group Prof. Dr. Karina Reiss

A Group Leader:

Prof. Dr. Karina Reiss



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Katharina Rothe

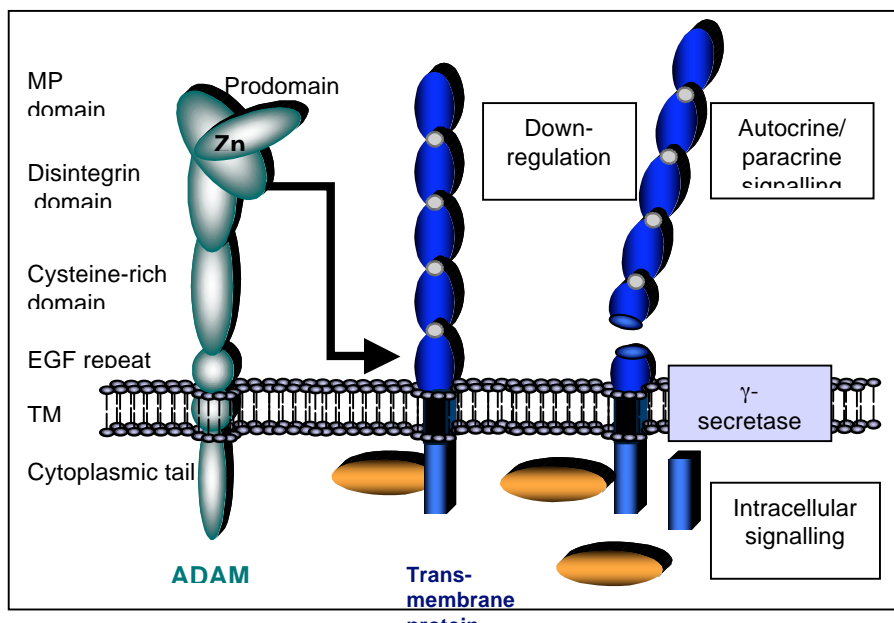
Laboratory Assistant:

Stefanie Jessen

Laboratory Assistant in training:

Tobias Lehmann
Meryem Senkara

C Research Report



C.1. Physiological functions of the metalloproteases ADAM 10 and ADAM17

ADAM10 is a member of the ADAM (A Disintegrin And Metalloprotease) family of single pass transmembrane proteins. ADAMs possess multiple functional domains: a prodomain, a disintegrin domain, the zinc binding metalloprotease domain, a cysteine rich domain, a EGF like domain, followed by a transmembrane domain and the cytoplasmic tail. Besides some hints for a role in neurogenesis and axonal pathfinding the physiological functions of this protease remain unclear. Currently, it is discussed as putative α -secretase cleaving the Amyloid Precursor Protein (APP) of Alzheimer's disease. While increasing numbers of potential *in vitro* substrates are identified the *in vivo* relevance remains mostly unclear. In order to examine the physiological role of this protease we generated ADAM10 deficient mice which die already at E 9.5 showing severe defects in heart development, neurogenesis and somitogenesis. Due to the embryonic lethality we studied the physiological role of this protease in ADAM 10 deficient fibroblast cell lines. While the processing of APP was mainly unaffected in knockout fibroblasts we found evidence for an important role of ADAM10 in the notch signalling pathway. Recently, we identified ADAM10 as sheddase responsible for the regulation of N-cadherin and E-cadherin cell surface expression, thereby regulating cell-cell adhesion and migration. Moreover, we could show that ADAM10 mediated cleavage of these adhesion molecules leads to redistribution of beta-catenin into the cytosol, increasing beta-catenin signalling and the expression of beta-catenin downstream genes like cyclin D1, leading to increased cell proliferation. Moreover, we could identify the neuronal cell adhesion molecule L1 as an ADAM10 substrate under constitutive conditions, while stimulated shedding was mediated by ADAM17. Therefore, our data indicate that ADAM10 is an important regulator of cell adhesion molecule functions. Besides the identification of putative ADAM10 substrates we also analyse the function of single ADAM10 domains by comparing chimeric proteases (combination with single ADAM17 domains). Additionally, we generated conditional ADAM10 knockout mice in cooperation with B. de Strooper (Leuven, Belgium) and Carl Blobel (New York, USA) in order to further characterise the physiological role of this important protease.

C.2. Physiological function of the aspartat protease BACE-1

Alzheimer's disease is characterized by the extracellular deposition of insoluble amyloid plaques. The main component of amyloid plaques which derives from a larger protein precursor (amyloid precursor protein, APP) is excised from APP by the sequential action of two protease activities known as β -secretase (BACE-1, Asp-2, memapsin 2), and the so called γ -secretase complex. The aspartat protease BACE-1 seems to be an ideal drug target for Alzheimer's Disease therapy and now inhibitors are being developed for therapeutic use. In view of this important role we developed mice deficient for BACE-1 to analyse the physiological role of this protease. In contrast to findings of other groups our knockout mice show increased postnatal mortality sometimes associated with pronounced weakness and multisystemic manifestations. Since this phenotype provides evidence for an important physiological role of BACE calling its perfect drug target function into question, we are now analyzing the cause of this maldevelopment and the physiological function of BACE-1.

D Publications 2008/2009

Publications 2008	Impact Factor
1. Raucci A, Cugusi S, Antonelli A, Barabino SM, Monti L, Bierhaus A, Reiss K, Saftig P, Bianchi ME (2008) A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). <i>FASEB J</i> 22:3716-27	7,0
2. Schulz B, Pruessmeyer J, Maretzky T, Ludwig A, Blobel C, Saftig P, Reiss K (2008) Disintegrin Metalloprotease (ADAM) 10 Regulates Endothelial Permeability and T Cell Transmigration by Proteolysis of Vascular Endothelial Cadherin. <i>Circ Res.</i> 102:1192-201	9,9
3. Maretzky T, Scholz F, Rudolph B, Proksch E, Saftig P, Reiss K (2008) The disintegrin-metalloproteinase 10 plays a key role for regulating keratinocyte cohesion in eczematous dermatitis. <i>J Invest Dermatol.</i> 128:1737-46	5,3
4. Reiss K and Saftig P (2008) The "A Disintegrin And Metalloprotease" (ADAM) family of Sheddases: Physiological and Cellular Functions. <i>Seminars in Cell and Developmental Biology.</i> 2:126-37.	4,5
5. Deuss M, Reiss K, Hartmann D (2008) Part time alpha-secretases: the functional biology of ADAM9, 10 and 17. <i>Curr. Alz. Res.</i> 5:187-201.	4,1

Publikationen 2009	Impact Factor
1. Bloch L, Sineshchekova O, Reichenbach D, Reiss K, Saftig P, Kuro-o M, Kaether C. (2009) Klotho is a substrate for alpha-, beta- and gamma-secretase. <i>FEBS Lett.</i> 583:3221-4	3,3
2. Steubesand N, Kiehne K, Brunke G, Pahl R, Reiss K, Herzig KH, Schubert S, Schreiber S, Fölsch UR, Rosenstiel P, Arlt A (2009) The expression of the beta-defensins hBD-2 and hBD-3 is differentially regulated by NF-kappaB and MAPK/AP-1 pathways in an in vitro model of Candida esophagitis. <i>BMC Immunol.</i> 10:36	2,7
3. Liu Q, Zhang J, Tran H, Verbeek MM, Reiss K, Estus S, Bu G (2009) LRP1 shedding in human brain: roles of ADAM10 and ADAM17. <i>Mol Neurodegener.</i> 4:17.	-
4. Foveau B, Ancot F, Leroy C, Reiss K, Vingtdoux V, Fafeur V, Tulasne D (2009) γ -secretase regulates MET tyrosine kinase receptor cleavages and HGF/SF-dependent transcriptional response. <i>Mol Biol Cell.</i> 20:2495-507	5,6
5. Koenen RR, Pruessmeyer J, Soehnlein O, Kenne E, Lindbom L, Fraemohs L, Schwarz N, Reiss K, Weber C, Ludwig A (2009) Regulated shedding of the junctional adhesion molecule JAM-A by the disintegrin and metalloproteinase ADAM17 controls neutrophil recruitment. <i>Blood.</i> 113:4799-809	10,4
6. Tousseyn T, Thathiah A, Jorissen E, Konietzko U, Reiss K, Maes E, Snellinx A, Serneels L, Nyabi O, Annaert W, Weskamp G, Blobel C, Saftig P, De Strooper B, Hartmann D (2009) Binding of TIMP1 induces regulated intramembrane proteolysis of ADAM10 and creates a nuclear signal involved in cell proliferation control. <i>J Biol Chem.</i> 284:11738	5,5
7. Le Gall S, Reiss K, Gibb DR, Conrad D, Saftig P, Blobel CP (2009) ADAMs 10 and 17 are differentially regulated components of the shedding machinery for TGF α , TNF α and other membrane proteins. <i>Mol Biol Cell.</i> 20:1785	5,6
8. Huth T, Schmidt-Neuenfeldt K, Rittger A, Saftig P, Reiss K, Alzheimer C. (2009) Non-proteolytic effect of beta-site APP-cleaving enzyme 1 (BACE1) on sodium channel function. <i>Neurobiol Dis.</i> 33:282-9	4,9

Impact factors 2008: 30.8

Impact factors 2009: 38

Total impact factors 2008/2009: 68.8

E Grants

- E.1. SFB415, Teilprojekt B9 "Functions of ADAM metalloproteases"; Paul Saftig, Karina Reiss
Fördersumme (2007 – 2010): 377.400,00 €
- E.2. Die Bedeutung von Disintegrin-ähnlichen Metalloproteasen (ADAMs) für epitheliale
Abwehrmechanismen ; SFB 617, assoziiertes Teilprojekt A26; Fördersumme 2007-2009: 77 000 €
- E.3. Exzellenzcluster Inflammation at Interfaces. Epithelial Protease Inhibitors (2008-2012); 628.900 €;
Miniproposal Fördersumme (2008) 50.000 €

7. Research Group PD Dr. Michael Schwake

A Group Leader: PD Dr. Michael Schwake

B Lab Members:



Doctoral students:

Michelle Danaher
Johann Groth
Christina Zachos
Christina Wehling (until 2009)

MD students

Miriam Wagner

Diploma students:

Judith Pohanke
Christian Raab
Daniel Milkereit (until 2009)

Bachelor students:

Friederike Zünke

Technicians:

Maike Langer
Katharina Stiebeling (until 2009)

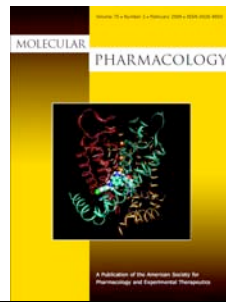
C Research Report

Epilepsy is a common neurological disorder that is characterized by recurrent unprovoked seizures. There are many different types of epilepsy that can be divided by presumptive cause. Inherited idiopathic epilepsies arise from mutations in different genes. The majority of those genes code for voltage-dependent and ligand-gated ion channels.

For example, mutations in the genes encoding the potassium channels *KCNQ2* and *KCNQ3* have been identified in several families with benign neonatal familial convulsions, an autosomal dominant epilepsy of infancy. These K^+ -channels contribute to the native muscarinic-sensitive K^+ -current that regulates excitability of numerous types of neurons and KCNQ channel activators such as retigabine are effective in epilepsy treatment. Based on structural models, biochemical experiments and electrophysiological measurements, we have characterised in recent years the binding site and the mode of action of retigabine on KCNQ channels. Interestingly, KCNQ2 and KCNQ3 form homomeric and heteromeric channels. In different recent studies we identified a domain, which specifically regulates the subunit-specific assembly of these channels. This domain comprises two coiled-coil domains, assembles into tetramers and probably directs the tetramerization of functional channel complexes.

In a similar context our group has recently shown that mutations in a lysosomal membrane protein (LIMP-2) are associated with a syndrome that is characterized by a focal glomerulosclerosis and progressive myoclonic epilepsy associated with accumulation of storage material in the brain. We could also show that LIMP2 and specifically a coil-coiled region within its luminal domain serves as a receptor for transporting glucocerebrosidase to the lysosome, an enzyme that specifically degrades glucocerebroside. Dysfunction of the enzyme leads to accumulation of the substrate in Gaucher disease, which is the most common inherited lysosomal storage disease and can be also associated with neurological dysfunction, such as seizures.

D Publications 2008/2009



Publications 2008	Impact Factor
1. Shuk Yin M Yeung, Schwake M., Pucovský V. and Greenwood I.A. (2008) Bimodal effects of the K _v 7 channel activator retigabine on vascular K ⁺ currents. Br J Pharmacol. 155, 62-72.	4.902
Publikationen 2009	Impact Factor
2. Lange W, Geissendörfer J, Schenzer A, Grötzinger J, Seebohm G, Friedrich T, Schwake M. (2009) Refinement of the binding site and mode of action of the anticonvulsant Retigabine on KCNQ K ⁺ channels. Mol Pharmacol. 75, 272-80.	4.711
3. Blanz J., Groth J., Wehling C., Saftig P. and Schwake M. (2009) Mutations within the lysosomal transport receptor lysosomal integral membrane protein type 2 (LIMP-2) reveal the nature of binding to β -glucocerebrosidase. Hum Mol Gent. Dec 3. [Epub ahead of print]	7.249

Impact factors 2008: 4,902

Impact factors 2009: 11,96

Total impact factors 2008/2009: 16,862

E Grants

- E.1. DFG, Graduiertenkolleg 1459, Sortierung und Wechselwirkung zwischen Proteinen subzellulärer Kompartimente, Fördersumme (2008-2012): 374.855 €
- E.2. DFG, Einzelantrag (SCHW866/4-1), Assemblierung und Transport von M-Strom vermittelnden Kv-Kanälen. Fördersumme (2008-2011): 126.100 €

8. Research Group Dr. Judith Blanz

A Group Leader:

Dr. Judith Blanz



B Lab Members:

Post-Doc

Alex Schneede (since 10.09)

MD students

Maike Lüdemann (until 03.09)

Technicians:

Inez Götting (2006-2009)

Trainee Lab. Technician

Meryem Senkara (since 06.09)

C Research Report

Within the last years the focus of my work is the study of i) preclinical Enzyme Replacement Therapy in mouse models for the Lysosomal Storage Disorder alpha-Mannosidosis, ii) the understanding of the mechanism that lead to neurodegeneration upon lysosomal dysfunction in Lysosomal Storage disorders (LSD) and iii) lysosomal function in general but specifically the function of the lysosomal membrane proteins LIMP-2 and LAMP2.

- 1) Enzyme Replacment Therapy in alpha-Mannosidosis mice within the EU granted project HUE-MAN (2006-2009).
- 2) Neuropathology of Lysosomal Storage Disorders especially alpha-Mannosidosis
- 3) Lysosomal Function of the lysosomal Membrane Proteins LIMP-2 and LAMP2

C.1. Preclinical Enzyme Replacment Therapy studies in a mouse model for alpha-Mannosidosis within the EU sponsored project HUE-MAN (2006-2009)

Lysosomal function depends on proper action of two classes of proteins, 1) lysosmal hydrolases that are responsible for lysosomal degradation and 2) lysosomal membrane proteins (LMP) that are involved in acidification of the lysosomal lumen, transport processes across the lysosomal membrane and lysosomal maturation. Lysosomal Storage Disorders (LSD) are a group of rare human genetic diseases in which a defect in lysosomal hydrolysis of macromolecules such as lipids and glycoproteins leads to intralysosomal accumulation of undegraded material This lysosomal dysfunction has severe consequences for the organism and many patients suffering from LSD die during early childhood. In brain, the accumulation of storage material often leads to neurodegeneration and inflammation. Treatment of LSD is hardly working since most of the diseases including alpha-Mannosidosis show severe neurological deficits. To date, Enzyme Replacement Therapy (ERT) is the most promising option for an efficient treatment of these diseases even though recombinant enzymes are thought not to be able to cross the the Blood Brain Barrier and therefore not to reach the central nervous system (CNS). In ERT, the respective enzyme lacking in the patient is produced by recombinant approaches and then introduced into the blood stream of the patient, from where it is internalized by the cells replacing the missing endogenous hydrolase.

Lysosomal accumulation of mannosyl-linked oligosaccharides leads to the orphan and devastating LSD alpha-Mannosidosis that is caused by deficiency of the lysosomal hydrolase alpha-Mannosidase (LAMAN). To date,

no real treatment for alpha-Mannosidosis is available. Since children are born healthy, an early initiated therapy shortly after birth could dramatically improve their life expectancy and quality of life. Since 2006, my group has worked towards developing the recombinant human enzyme (rhLAMAN) as a therapeutic agent for patients suffering from alpha-Mannosidosis together with European scientists and clinicians all over Europe within the EU sponsored project HUE-MAN. Within this project, we developed an efficient preclinical ERT protocol for treatment of alpha-Mannosidosis in mice. Alpha-Mannosidosis mice that have been used in these studies were obtained by targeted disruption of the LAMAN gene and have been shown to be a valid mouse model for the human disease.

Upon frequent injections of the human recombinant enzyme, alpha-Mannosidosis mice developed humoral immune responses associated with high mortality. Therefore, our ERT studies were limited to a maximum of four injections within two weeks. After intravenous injection, rhLAMAN was widely distributed among tissues and immunohistochemistry revealed lysosomal delivery of the injected enzyme, indicating correct targeting. Dose finding studies revealed that storage in visceral tissues was cleared (>70%) after two injections of low doses (25U-100U/kg) whereas in brain higher doses were needed. Doses of 250U/kg were sufficient for clearance of stored substrates in peripheral neurons of the trigeminal ganglion whereas high dose treatment with four injections of 500U/kg led to a >50% clearance of sugar storage in brain. Successful transfer across the blood-brain barrier was evident, since the injected enzyme was found in hippocampal neurons, leading to a nearly complete disappearance of storage vacuoles within these neurons. However, the sugar reduction was not permanent since 12 days after the last injection, sugars in brain start to accumulate again. The clearance of sugar storage was demonstrated by various methods such as morphological analysis (disappearance of storage vacuoles) and chromatographical methods (Thin Layer Chromatography and HPLC of sugar tissue extracts). A morphological analysis of mouse brains before and after ERT and Thin Layer Chromatography (TLC) of brain sugar extracts, respectively, is shown in Figure 1.

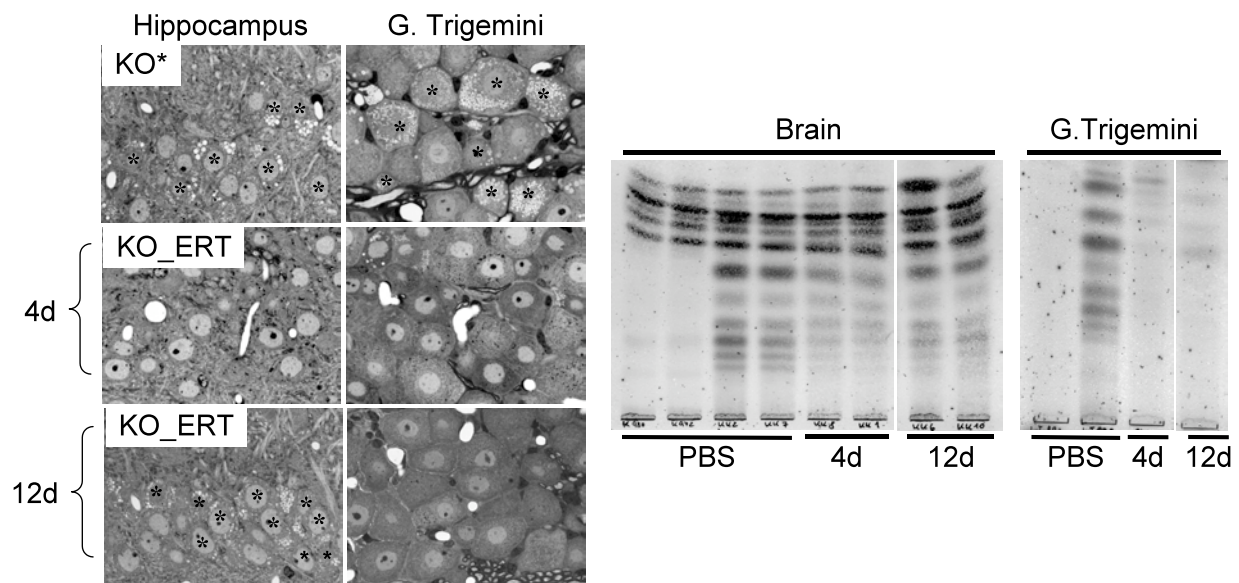


Figure 1: Immunohistology (left panel) of brain sections and TLC analysis (right panel) of sugar extracts obtained from brains (CNS) and the *G.trigemini* (PNS) from KO mice before (KO*) and after treatment (KO_ERT). Mice have been treated 4x with high doses of rhLAMAN (500U/kg) and were analysed 4, 8 and 12 days (d) after the last injection.

Our data suggest that rhLAMAN must be able to cross the Blood Brain Barrier but the mechanism how rhLAMAN enters the brain remains unclear and needs further investigations. The successful outcome of the HUE-MAN projects are now the base for clinical trials that we hope to realize within a third EU granted project (ALPHA-MAN). Together with the Scandinavian company Zymenex that developed rhLAMAN as a therapeutic product we set up an EU application for ALPHA-MAN that was submitted November 2009 and will be evaluated by March 2010.

C.2. Generation and Characterization of an immuntolerant alpha-Mannosidosis mouse model.

The disadvantage using alpha-Mannosidosis mice for ERT is that they develop a severe immune-reaction after frequent injections of the human LAMAN enzyme precluding long-term ERT. Since chronic dosing studies will help us to find i) the mechanism by which the enzyme crosses the Blood Brain

Barrier and ii) to see, to which extent, the observed neuropathology (see section D2) in alpha-Mannosidosis mice is reversible, we generated a transgenic mouse model, that expresses an inactive form of the human LAMAN within the alpha-Mannosidase KO background (Tg^{H72L}/mLAMAN KO). The idea is, that a mouse model, that is deficient for the mouse LAMAN, but expresses an inactive form of the human enzyme, shows the “classical” KO phenotype but tolerates the injected human enzyme, allowing chronic ERT studies. This approach has already been successfully used to generate an immune-tolerant mouse model for the LSD “Metachromatic Leukodystrophy”. Indeed, first ERT experiments that have been carried out with Tg^{H72L}/mLAMAN KO mice suggest that these mice are indeed immunetolerant to the injected recombinant human enzyme (*unpublished data*). These mice can now be used for further long term studies and help us to investigate the *in vivo* mechanism by which the enzyme crosses the Blood Brain Barrier and to see, to which extent, the observed neuropathology in alpha-Mannosidosis mice is reversible upon long term dosing.

C.3. Neuropathology of the Lysosomal Storage Disorder alpha-Mannosidosis

To better understand the efficacy of ERT in terms of the underlying neuropathology we are currently investigating brains of alpha-Mannosidosis mice for a neurological phenotype which has not been described so far. Our biochemical and histological analyses performed revealed a distinct and specific neuropathology in the cerebellum of alpha-Mannosidosis mice. In addition to the well characterized sugar storage in brain of alpha-Mannosidosis mice, we found an accumulation of free cholesterol and Gangliosides (GM1) specifically in the molecular layer of the cerebellum that is associated with macrophage infiltration, regional restricted astrogliosis and partial loss of Purkinje cells (*unpublished data*). The observation of the neuropathological abnormalities in alpha-Mannosidosis mice is very important since they can now be used as a “clinical endpoint” to study the efficacy of ERT in the CNS of alpha-Mannosidosis mice. In addition, it will help us to understand the link between primary sugar storage and underlying behavioural deficits.

C.4. Lysosomal Function of the lysosomal Membrane Protein LIMP-2.

The lysosomal membrane is important for proper lysosomal function. One of the most abundant lysosomal membrane proteins is the lysosome integral membrane protein 2 (LIMP-2) that was recently identified as a disease causing gene for the Action Myoclonus Renal Failure Syndrome (AMRF). AMRF is a rare kidney disease associated with epilepsy and ataxia caused by mutations in LIMP-2 that lead to a deficiency of the LIMP-2 protein. To correlate the consequences of LIMP2 deficiency in mice and men we performed detailed morphological analyses of LIMP-2 deficient mice and compared the phenotype with the pathological changes that were found in *post mortem* material of AMRF patients. AMRF patients as well as LIMP-2 deficient mice show intracellular inclusions in cerebral and cerebellar cortex and subtle glomerular changes in the kidney. In addition, both suffer from ataxia which implies dysfunction of the cerebellum possibly as a consequence of the accumulated storage within this brain structure. Our data suggest that LIMP-2 deficient mice can serve as a mouse model for AMRF and can now be used to further investigate the AMRF neuropathology. These studies will be essential in clarifying the role of LIMP-2 in brain that is still elusive.

In 2007, LIMP-2 was identified as a receptor for targeting the lysosomal hydrolase β -Glucocerebrosidase (β GC) to the lysosome. LIMP-2 is a type II transmembrane protein, spanning the membrane twice with a large luminal domain. *In vitro* binding assays suggested that the binding of β GC to LIMP-2 occurs within the luminal domain of LIMP-2 and that a putative coiled-coiled motif from amino acid 152-167 within this domain is important for this interaction. To better understand the nature of the interaction between LIMP-2 to β GC we have analyzed most of the known AMRF disease causing mutations of LIMP-2 and the resulting truncated proteins in more detail. To date, six AMRF-causing mutations have been described, including splice site, missense and nonsense mutations. All mutations we have investigated lead to a retention of LIMP-2 in the endoplasmic reticulum (ER) but affect the binding to β GC differentially. For studying the binding of β GC to LIMP-2 we have used the immunoprecipitation technique. Using this approach we were able to narrow down the binding site to a region between amino acid 145 and 288 within the luminal domain of LIMP-2. The LIMP-2 segment 145–288 also contains the highly conserved coiled-coil domain, which we suggest determines β GC binding. In fact, disruption of the helical arrangement and amphiphatic nature of the coiled-coil domain abolishes β GC binding, and a synthetic peptide comprising the coiled-coil domain of LIMP-2 displays pH-selective multimerization properties. Our data clearly demonstrate that the disruption of the coiled-coil structure or AMRF disease-causing mutations abolish β GC binding, indicating the importance of an intact coiled-coil structure for the interaction of LIMP-2 and β GC. However, further structural analyses of LIMP-2 and β GC complexes are needed to understand their molecular interaction in more detail.

D Publications 2008/2009

Publications 2008	Impact Factor
1. Blanz J, Stroobants S, Lüllmann-Rauch R, Morelle W, Lüdemann M, D'Hooge R, Reuterwall H, Michalski JC, Fogh J, Andersson C, Saftig P. (2008) Reversal of peripheral and central neural storage and ataxia after recombinant enzyme replacement therapy in alpha-mannosidosis mice. <i>Hum Mol Genet.</i> 17, 3437-45.	7.249
2. Berkovic SF, Dibbens LM, Oshlack A, Silver JD, Katerelos M, Vears DF, Lüllmann-Rauch R, Blanz J, Zhang KW, Stankovich J, Kalnins RM, Dowling JP, Andermann E, Andermann F, Faldini E, D'Hooge R, Vadlamudi L, Macdonell RA, Hodgson BL, Bayly MA, Savige J, Mulley JC, Smyth GK, Power DA, Saftig P, Bahlo M. (2008) Array-based gene discovery with three unrelated subjects shows SCARBsLIMP2-deficiency cause myoclonus epilepsy and glomerulosclerosis <i>Am J Hum Genet.</i> 82, 673-84.	10.153
Publications 2009	Impact Factor
1. Blanz J, Groth J, Zachos C, Wehling C, Saftig P, Schwake M. (2009) Disease causing mutations within the lysosomal intergral membrane protein type 2 LIMP-2 reveal the nature of binding to its ligand β -glucocerebrosidase. <i>Hum Mol Genet.</i> 2009 Nov 20.	7,249
2. Schneede A, Schmidt CK, Hölttä-Vuori M, Heeren J, Willenborg M, Blanz J, Domansky M, Breiden B, Brodesser S, Landgrebe J, Sandhoff K, Ikonen E, Saftig P, Eskelinen EL. (2009) A Role for LAMP2 in endosomal Cholesterol transport. <i>J Cell Mol Med.</i> 2009 Nov 19.	5.114

Impact factors 2008: 17.40

Impact factors 2009: 12.36

Total impact factors 2008/2009: 29.77

E Grants

Currently no grants

9. Research Group Dr. Bernd Schröder

A Group Leader:

Dr. rer. Physiol. Bernd Schröder



B Lab Members:

Doctoral Students:

Janna Schneppenheim

Diploma Students:

Jörg Behnke

Nur Güneli

Laboratory Assistant:

Sebastian Held

Laboratory Assistant in training:

Raffael Kurz

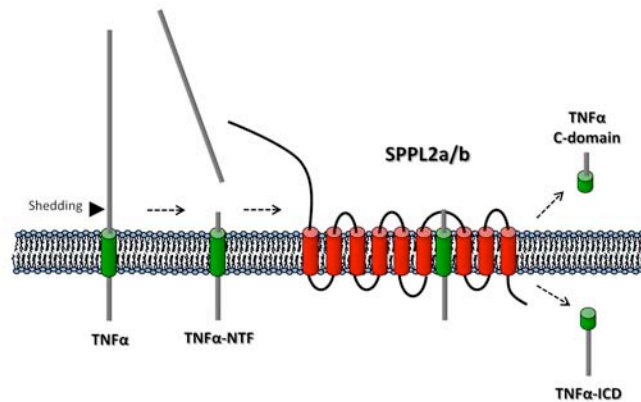
C Research Report

C.1. Physiological functions of the intramembrane proteases SPPL2a and SPPL2b

The concept of regulated intramembrane proteolysis (RIP) has emerged over the last decades as a novel concept of cellular signalling. One group among the proteases being capable of cleaving substrates within the phospholipid bilayer are the signal-peptide-peptidase (SPP) and its homologues, the signalpeptide-peptidase-like proteins (SPPL2a, -2b, -2c, -3). Whereas SPPL2a is present in membranes of lysosomes/late endosomes, SPPL2b was reported to reside at the plasma membrane as well as in endosomal compartments. To date, only TNF α , Fas Ligand (FasL) and Bri2 (Itm2b) have been identified as substrates of SPPL2a/b using *in vitro* overexpression approaches. In agreement with the general concept of RIP it was demonstrated that the TNF α intracellular domain translocates into the nucleus after proteolytic release and influences gene expression thereby inducing the synthesis of the pro-inflammatory cytokine IL12. Two of the known substrates suggest a regulatory function of SPPL2a and SPPL2b in the context of the immune system. However, a relevance of these processes and proteolytic events in a complex *in vivo* system has not been analysed and established yet. It was also suggested that RIPping by SPPL2a and SPPL2b may have more generalized degradative functions beyond mediating reverse signalling by TNF α or other members of this superfamily - a hypothesis being supported by the presence of SPPL orthologues in plants.

In order to study functions of SPPL2a and SPPL2b *in vivo*, we have generated mouse lines deficient in either of the two proteases as well as mice being deficient in SPPLa and SPPL2b. Phenotypic analysis of these mice has revealed so far major immunological abnormalities associated with the deficiency of SPPL2a. These findings point to a fundamental role of SPPL2a mediated proteolysis in immunity that cannot be compensated for by SPPL2b.

These *in vivo* approaches are combined with biochemical and cell biological experiments studying the differential processing of known and putative substrates in SPPL2a/b deficient cell lines.



We are confident, that challenging these mice in infection and inflammation models will contribute to understanding the role of SPPL2a/2b-mediated RIPping in pathophysiology and deepen our understanding of cellular and molecular principles of inflammation. The results of these studies will help us to decide whether SPPL2a and/or -2b might even be considered as potential drug targets and if specific inhibitors of these proteases might be capable of modulating the immune system in a therapeutic way. A central objective of the project is the unbiased search for novel substrates cleaved by SPPL2a and/or -b and to ideally link the biochemical findings with the phenotypes observed in the protease deficient mice.

C.2 Functional characterisation of novel lysosomal membrane proteins

Lysosomes play a crucial role in the degradation and turnover of different intra- and extracellular macromolecules. Currently, extensive data are available about the proteins of the lysosomal matrix. On the contrary only a minority of the lysosomal membrane proteins has been identified and substantially characterised to date. This contrasts with the number of known and functionally described transport systems or enzymatic activities that have been shown to be associated with this membrane.

In a previous proteomic analysis of lysosomal membranes we have identified 16 novel enzyme and transporter proteins and 12 novel proteins of unknown functions not previously assigned to lysosomal membranes. Lysosomal localisation of several of these novel proteins could be confirmed by overexpression studies. Current work focusses on an in-depth biochemical characterisation of a selection of these proteins and will be complemented by the generation of knock-out mice in order to unravel the functions of these novel lysosomal membrane proteins.

D Publications 2008/2009

Publications 2008	Impact Factor
1. Beertsen,W., Willenborg,M., Everts,V., Ziropianni,A., Podschun,R., Schröder,B., Eskelinen,E.L., and Saftig,P. (2008). Impaired phagosomal maturation in neutrophils leads to periodontitis in lysosomal-associated membrane protein-2 knockout mice. <i>J. Immunol.</i> 180, 475-482.	6.000
Publikationen 2009	Impact Factor
1. Schröder,J., Lüllmann-Rauch,R., Himmerkus,N., Pleines,I., Nieswandt,B., Orinska,Z., Koch-Nolte,F., Schröder,B., Bleich,M., Saftig,P. (2009). Deficiency of the tetraspanin CD63 associated with kidney pathology but normal lysosomal function. <i>Moll.Cell Biol.</i> 29(4), 1083-1094.	5.942

2. Schieweck,O., Damme,M., Schröder,B., Hasilik,A., Schmidt,B., Lübke,T. (2009). NCU-G1 is a highly glycosylated integral membrane protein of the lysosome. <i>Biochem. J.</i> 422(1), 83-90.	4.371
3. Hasilik,A., Wrocklage,C., Schröder,B. (2009). Intracellular trafficking of lysosomal proteins and lysosomes. <i>Int J Clin Pharmacol Ther</i> 47, 18-33.	1.299

Impact factors 2008: 6.0

Impact factors 2009: 11.6

Total impact factors 2008/2009: 17.6

E Grants

Currently no grants

10. Research Group Prof. Dr. Ursula Just

A Group Leader:

Prof. Dr. Ursula Just



B Lab Members:

Group leaders:

Dr. Thomas Höfken

Dr. Ralf Schwanbeck

Post-Doc:

Dr. Franziska Meier-Stiegen
(until June 08)

Doctoral Students:

Franziska Meier-Stiegen (until Feb. 08)

Simone Martini

Kristina Bernoth

Meng Lin

Christopher Tiedje

Diploma Students:

Mingfei Cui

Junie Tchudjin

Technician:

Melanie Boss

Silke Horn

Beatrix Berger

Secretary:

Petra Voß

C Research Report

C.1. Retroviral insertional mutagenesis as a route to identifying unknown regulatory genes involved in self renewal

Non-leukaemic murine hematopoietic stem cell lines (called Factor-Dependent-Cell-Paterson-mixed potential) have been isolated, that possess many characteristics of very immature haematopoietic progenitor cells. These include the short-term repopulation of hematopoiesis and the formation of spleen colonies in irradiated mice, establishment of long-term haematopoiesis on mouse stroma, the ability of self-renewal in the presence of interleukin-3 (IL-3), and the ability to differentiate in a multilineage response to haematopoietic growth factors

and to haematopoietic stromal cells (Sponcer et al., *Differentiation* 31, 111-118, 1986; Just et al., *Curr. Top. Microbiol. Immunol.* 25, 27-34, 2000). In contrast to normal hematopoietic stem cells, FDCP-mix cells have an increased self renewal capacity in vitro: While normal, uninfected long term cultures of hematopoietic stem cells gradually lose their self renewal capacity after several passages on normal mouse bone marrow stroma, the balance of self renewal versus differentiation is shifted in favour of self-renewal in FDCP-mix cells (Just et al., *Curr. Top. Microbiol. Immunol.* 25, 27-34, 2000 and unpublished). FDCP-mix cells were generated by retroviral insertional mutagenesis. Using the retroviral integration sites as a tag (U. Just et al., unpublished) as well as genome wide gene expression profiling (U. Just et al., unpublished), several candidate genes for the increased self renewal capacity of FDCP-mix cells were identified. Among them were H3K4 methyltransferases and a H3K4 demethylases. Cell identity is in large part regulated at the level of chromatin structure, with several key developmental regulators acting as modifiers of histones. Numerous histone modifications can be correlated with particular transcriptional states of a gene and in distinct areas within the genome. Among the best characterized modifications are the methylation of histone H3 on lysine 4 and 27 (H3K4 and H3K27), regulated by methyltransferases and demethylases and their related protein complexes. H3K4 methylation occurs at the promoters of actively expressed genes, whereas H3K27 methylation is associated with the silencing of genes during development. Repression of a subset of genes within adult stem cells is required to maintain stem cell identity, and their premature activation results in stem cell loss (Buszak et al., *Science* 323, 248-251, 2009). Interestingly, all FDCP-mix cell lines show either increased expression of H3K4 demethylases or/and decreased expression of H3K4 methyltransferases, most likely resulting in decreased H3K3 methylation, which, as in *Drosophila*, may be required for maintaining the increased self renewal capacity of FDCP-mix cells, i.e. the stem cell identity. Currently, we are analysing the role of these chromatin modifying enzymes by overexpression and knock down of the expression using siRNA in FDCP-mix cells and primary murine and human HSC, respectively. In collaboration with Prof. R. Huss, Roche, known inhibitors and activators as well as a high-throughput screening of small-molecule libraries will be tested for increasing self renewal of FDCP-mix cells, and in the next step of murine and human HSC.

C.2. Role of the Notch pathway in mesodermal development and kidney disease

Intracellular signalling through the Notch transmembrane receptors regulates proliferation and differentiation in many developmental systems. Notch is activated by binding a member of the Delta and Serrate/Jagged family of cell-surface proteins. Following activation, Notch is cleaved within the transmembrane domain, releasing the Notch intracellular domain (NIC) from the membrane. The NIC then translocates to the nucleus where it can modulate gene expression via association with CSL proteins (RBP-J in mammals), and thereby affect cell fate choice. Notch receptors and cognate ligands are expressed throughout development. To analyse the role of Notch signaling in the development of mesoderm-derived cell lineages and in particular of hematopoietic cells, we expressed a constitutive active form of murine Notch 1 in embryonic stem cells (ES) and in the multipotential hematopoietic cell line FDCP-mix using a Tamoxifen-inducible expression system and looked at how activated Notch1 affects lineage commitment, differentiation and proliferation of the cells. We found that Notch signaling plays a regulatory role in mesodermal development, cardiomyogenesis, hematopoietic development and the balanced generation of blood vessel cell types (Schroeder and Just, *EMBO J.* 19, 2558-2568, 2000; Schroeder et al., *PNAS* 100, 4018-4023, 2003; Schroeder et al., *Mech. Dev.* 123, 570-579, 2006; Yurugi-Kobayashi et al., *Arterioscler. Thromb. Vasc. Biol.* 26, 1977-1984, 2006; Henning et al., *Cell Death Differ.* 15, 398-407, 2008). To elucidate the molecular mechanisms by which Notch influences cell lineage decisions in a cell context dependent manner, we have identified cell-context dependent Notch target genes (see also research report of Dr. Ralf Schwanbeck). Interestingly, Notch directly upregulated transcription factors involved in cell lineage decisions (Schwanbeck et al., *Cells Tissues Organs* 188, 91-102, 2008, and Meier-Stiegen et al., submitted). In collaboration with Prof. U. Lendahl, Karolinska Institute, Sweden, we are currently analysing the function of Notch-mediated upregulation of these specific transcription factors for cell lineage decisions during mesodermal and ectodermal development.

In response to injury, the kidney reactivates embryonic developmental programs, among them the Notch signaling pathway, which plays an essential role during early nephrogenesis, glomerulogenesis and tubulogenesis. In diabetic nephropathy, Notch signaling is activated in podocytes and leads to destruction of the glomerular barrier. Notch signaling is further activated after ischemia in acute tubular necrosis. In acute as well as chronic renal failure, tissue hypoxia is an important pathomechanism. Notch and hypoxia interact at the molecular level by the induction of common target genes. Several of the Notch target genes we identified in our system are also activated under hypoxia or by combined hypoxia and Notch activation (collaboration with Prof. U. Lendahl, Karolinska Institute, Sweden). In collaboration with Prof. Rohwedel and Dr. Kramer we are currently testing in a mouse model of ischemic kidney failure which Notch and hypoxia target genes are activated, to get further insight into the pathogenesis of kidney failure.

C.3. Gene regulation by the Notch pathway in stem cells

See Research Report of Dr. Ralf Schwanbeck

C.4. HMGA proteins in stem cells and cancer gene regulation

See Research Report of Dr. Ralf Schwanbeck

C.5. Regulation of cell polarity by Cdc42 and its effectors:

C.5.1. PAKs and sterol homeostasis

C.5.2. Regulation of Rho GTPases by the Rho GDI Rdi1

See Research Report of Dr. Thomas Höfken

D Publications in 2008/2009

Publications 2008	Impact Factor
1. Schwanbeck, R., Schroeder, T., Henning, K., Kohlhof, H., Rieber, N., Erfurth, M. L., Just, U. Notch Signaling in Embryonic and Adult Myelopoiesis. (2008) Cells Tissues Organs 188: 91-102.	2.376
2. Henning, K., Heering, J., Schwanbeck, R., Schroeder, T., Helmbold, H., Schaefer, H., Deppert, Kim, E., Just, U. Notch1 activation reduces proliferation in the multipotent hematopoietic progenitor cell line FDCP-mix through a p53-dependent pathway but Notch1 effects on myeloid and erythroid differentiation are independent of p53.(2008) Cell Death Differ. 15, 398-407.	7.548
3. Just, U., Cross, M. Stem cells, tissue regeneration and repair. (2008) Cells Tissues Organs 188, 5.	2.376
4. Tiedje C, Sakwa I, Just U, Höfken T. (2008) The Rho GDI Rdi1 regulates Rho GTPases by distinct mechanisms. Mol. Biol. Cell 19:2885-2296.	5.558
Publikationen 2009	Impact Factor
1. Lin M, Unden H, Jacquier N, Schneiter R, Just U, Höfken T. (2009) The Cdc42 effectors Ste20, Cla4, and Skm1 down-regulate the expression of genes involved in sterol uptake by a mitogen-activated protein kinase-independent pathway. Mol. Biol. Cell. 20:4826-4837.	5.558
2. Lin M, Grillitsch K, Daum G, Just U, Höfken T. (2009) Modulation of sterol homeostasis by the Cdc42p effectors Cla4p and Ste20p in the yeast Saccharomyces cerevisiae. FEBS J. 276:7253-7264.	3.421

Impact factors 2008: 17,858

Impact factors 2009: 8.979

Total impact factors 2008/2009: 26.837

E GRANTS

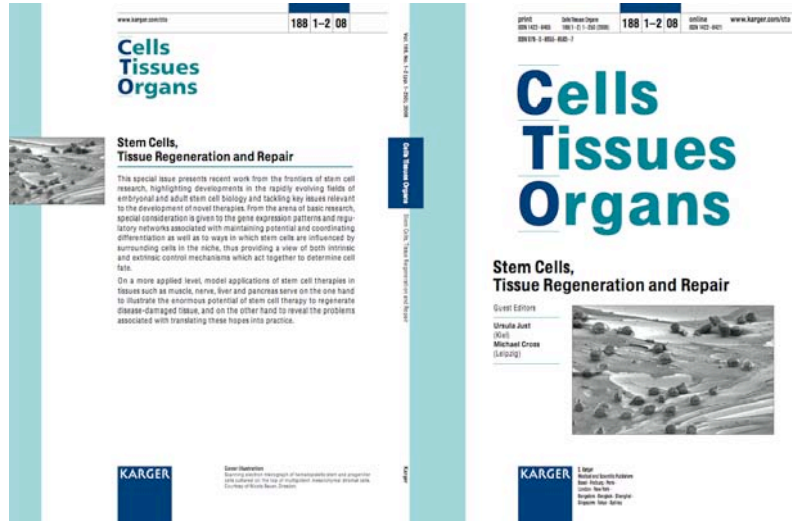
E.1. Sonderforschungsbereich 415: "Spezifität und Pathophysiologie von Signaltransduktionswegen"
Teilprojekt B8: 'Characterization of Notch/RBP-J signalling in dependence of the cellular context during mesodermal differentiation', Fördersumme (2007 – 2010) 241.200 €

E.2. Hensel-Stiftung
Analyse der Rolle des Notch-Signaltransduktionswegs beim Nierenversagen, Fördersumme (ab 2009) 30.000 €

F FUNCTIONS

Member of the National Ethics Committee for Stem Cell Research (ZES) since July 2005

Editor for the DFG special issue on Stem Cells, Tissue Regeneration and Repair:



11. Research Group Dr. Thomas Höfken

A Group Leader:

Dr. Thomas Höfken



B Lab Members:

Doctoral students:

Meng Lin
Christopher Tiedje

Diploma students:

Mingfei Cui
Junie Tchudjin

Technician:

Melanie Boß
Silke Horn

C. Research Report

The generation of cell polarity is critical for the control of many biological processes such as early embryogenesis, intracellular transport of organelles in polarized epithelial cells, directed movement of migratory cells, neurite outgrowth and asymmetric cell division. We use the budding yeast *Saccharomyces cerevisiae* as a model organism to study the biochemical and molecular biological mechanisms underlying cell polarity. The membrane-bound Rho-type GTPase Cdc42 plays a key role in the establishment and maintenance of cell polarity. Like other small GTPases, Cdc42 is either associated with GDP and inactive or in a GTP-bound active stage. Active Cdc42 in turn recruits effectors such as the p21-activated kinases (PAKs) Ste20, Cla4 and Skm1 from the cytosol and activates them. Cdc42 effectors signal to the actin cytoskeleton inducing polarized arrangements of actin, but also have other functions in polarity. However, exact molecular mechanisms of these effectors are only partly understood. The activity of Cdc42 is regulated by GEF, GAP and GDI proteins. Our group is interested in two aspects of cell polarity, the regulation of Cdc42 activity and the function of Cdc42 effectors

C.1. PAKs and sterol homeostasis

The conserved PAK-family kinase and Cdc42 effector Ste20 is involved in various aspects of cell polarity. In order to identify novel regulators and downstream targets of this kinase, we carried out a split-ubiquitin screen, a modified two hybrid screen that is particularly suitable for membrane-associated proteins. Among other proteins, we obtained several proteins involved in sterol homeostasis (sterol biosynthesis, storage and uptake). These interactions were confirmed using pull-down assays. Interestingly, the deletion of genes involved in sterol synthesis (*ERG4*, *NCPI* and *CBRI*) resulted in various polarity defects. Since the reactions catalyzed by Ncp1 and Cbr1 are major regulatory steps of sterol biosynthesis it is tempting to speculate that Ste20 regulates sterol

synthesis. Indeed, deletion of either *STE20* or *CLA4* results in increased sterol levels, suggesting that these Cdc42 effectors negatively regulate sterol biosynthesis.

To keep sterol levels constant, sterols are stored as steryl esters in cytosolic lipid particles. Cla4 down-regulates steryl ester formation by inhibiting Are2, the major enzyme catalyzing this reaction. Furthermore, cells lacking Are2 have a defect in polarized bud growth.

We have shown that Ste20, Cla4 and Skm1 not only localize to the plasma membrane at sites of polarized growth but also translocate into the nucleus. There they interact with Sut1, a transcriptional regulator involved in sterol uptake. Ste20, Cla4 and Skm1 down-regulate the expression of the Sut1 target genes *AUS1* and *DAN1*, which promote sterol uptake. The fact that Ste20 and Cla4 regulate so many different aspects of sterol homeostasis suggests that this plasma membrane lipid has a crucial role in the establishment and maintenance of cell polarization.

The only function attributed to the transcriptional regulator Sut1 to date is sterol uptake. We could also show that Sut1 regulates cell polarization by a mechanism that is independent of sterol uptake. Sut1 controls the expression of polarity genes. A detailed characterization is in progress.

C.2. Regulation of Rho GTPases by the Rho GDI Rdi1

In addition to GEFs and GAPs, Rho-type GTPases are also regulated by GDIs (guanine nucleotide dissociation inhibitors). These proteins are able to extract Rho proteins from membranes by binding to the hydrophobic isoprenyl group of Rho proteins that serves as a membrane anchor. It has been proposed that in response to some stimuli the inactive cytosolic GDI-GTPase complex could be targeted to specific membrane domains where it activates other cell polarity proteins. However, very little is known about Rho GDIs.

We demonstrated that Rdi1, the only Rho-GDI in budding yeast, interacts only with Cdc42, Rho1 and Rho4, but not with other Rho GTPases. The PAK-family kinase Cla4 disrupts Rdi1-Cdc42 and Rdi1-Rho1 complexes. Since Cla4 also acts downstream of Cdc42, both proteins may constitute a positive feedback loop in the establishment of cell polarity. Interestingly, Rho4 is degraded following membrane extraction by Rdi1. This unusual way of regulation depends on GSK-3 β , the proteasom and vacuolar/lysosomal proteases.

To identify potential regulators of Rdi1, we performed a split-ubiquitin screen using Rdi1 as bait. 4 proteins isolated in this screen are of particular interest because they localize to sites of polarized growth. We currently characterize these proteins.

D. Publications 2008/2009

Publikationen 2008	Impact Factor
1. Tiedje C, Sakwa I, Just U, Höfken T (2008) The Rho GDI Rdi1 regulates Rho GTPases by distinct mechanisms. <i>Mol. Biol. Cell</i> 19:2885-2296	5.558
Publikationen 2009	Impact Factor
1. Lin M, Unden H, Jacquier N, Schneiter R, Just U, Höfken T (2009) The Cdc42 effectors Ste20, Cla4, and Skm1 down-regulate the expression of genes involved in sterol uptake by a mitogen-activated protein kinase-independent pathway. <i>Mol. Biol. Cell</i> . 20:4826-4837	5.558
2. Lin M, Grillitsch K, Daum G, Just U, Höfken T (2009) Modulation of sterol homeostasis by the Cdc42p effectors Cla4p and Ste20p in the yeast <i>Saccharomyces cerevisiae</i> . <i>FEBS J.</i> 276:7253-7264.	3.421

Impact factors 2008: 5.558

Impact factors 2009: 8.979

Total impact factors 2008/2009: 14.537

E. Grants

E.1. Characterization of the potential Cdc42 regulators Dop1 and Rdi1 in budding yeast
DFG HO2098/2-1, Fördersumme (2005-2008) 254.000 €

E.2. Regulation of cell polarity by control of Sut1-mediated gene expression via the Cdc42 effectors Ste20, Cla4 and Skm1
DFG HO2098/5 Fördersumme (2009-2011) 160.000 €

12. Research Group Dr. Ralf Schwanbeck

A Group Leader:

Dr. Ralf Schwanbeck



B Lab Members:

Doctoral Students:

Simone Martini (Co-supervised with Ursula Just)

Kristina Bernoth (Co-supervised with Ursula Just)

Technician:

Beatrix Berger

Silke Horn

C Research Report

C.1. Gene regulation by the Notch pathway in stem cells

Cell-cell communication is a crucial mechanism in organisms to orchestrate the temporal and spatial gene expression patterns during development. The stem cells we investigate have the ability to differentiate to various or - in the case of embryonic stem cells - to all different cells types and tissues occurring in an organism. The complex mechanism underlying that differentiation process is just in the beginning to be understood.

One system that is investigated in our group is the cell lineage decision by the Notch signaling. Notch belongs to highly conserved transmembrane glycoprotein receptors that regulate self renewal, differentiation, proliferation and apoptosis of embryonic and adult stem cells. Specific transmembrane ligands encoded by the Delta and Serrate/Jagged family activate Notch receptors on neighboring cells, inducing proteolytic liberation by ADAM and Presenilin/g-secretase proteases and nuclear translocation of the intracellular domain of Notch (NIC). Nuclear NIC associates with the transcriptional repressor RBP-J, converting it from a repressor into an activator by replacing a corepressor complex with a coactivator complex containing histone acetyltransferases. Our studies revealed that activated Notch1 alters differentiation of embryonic stem cells into mesodermal cell lineages at multiple stages of development. Thus, the activation of Notch1 inhibits differentiation into mesodermal cells and promotes differentiation along the neuronal lineage in ES cells as well as it inhibits the generation of cardiac muscle, endothelial and hematopoietic cells and favors the generation of mural cells in mesodermal progenitor cells. Furthermore, we have identified Notch target genes at several stages during embryonic development and in adult hematopoietic cells. For embryonic and mesodermal progenitor cells we used the Affymetrix Mouse Genome Array that covers the whole genome. In embryonic stem cells, Notch/RBP-J signaling alters the expression of 118 genes under culture conditions that allow ectodermal differentiation and 137 genes under culture conditions that restrict ectodermal differentiation but allow mesodermal differentiation. In mesodermal flk⁺ progenitor cells, 262 genes are regulated. Interestingly, Notch signaling activates mostly different genes in different culture conditions and at different stages of development, underscoring the importance of the cell context dependency of Notch signaling. Furthermore, experiments using the protein synthesis inhibitor cycloheximide revealed direct target genes of the Notch pathway. Comparing the differentially expressed genes in mesodermal progenitor cells with and without CHX revealed a significant overlap for the up-regulated fraction, whereas there is almost no overlap for the down-regulated genes. This suggests that positive regulation is the main mechanism of activated Notch1, whereas the negative regulation observed accounted for indirect effects.

Recent projects investigate the Notch specific chromatin remodeling effects on selected target promoters to understand the gene regulatory activity on a molecular level. Preliminary experiments suggested that particular chromatin marks are a prerequisite for Notch activation and that regulatory regions of Notch target genes are substantially remodeled after induction. This may serve as a model for the cell-context specificity of Notch signaling.

In hematopoietic stem cells the activation of Notch leads to a p53 dependent upregulation of p21 - a downstream mediator of p53-induced growth arrest. This effect seems not to be RBP-J-dependent. p21 was shown to be a direct Notch target gene in tissues and tumors where Notch has an antiproliferative effect. Our data showing the strict dependency of the Notch-p21 induced growth arrest on p53 suggest that the p53 status is critical for tumor development in tissues in which Notch and p21 play a role.

C.2. HMGA proteins in stem cells and cancer gene regulation

The High Mobility Group (HMG) proteins are abundant non-histone proteins that are able to bind DNA and nucleosomes and induce structural changes in the chromatin fiber. The HMGA protein family contains three AT-hook motifs, enabling the binding into the minor groove of AT-rich DNA. This binding, which is rather DNA structure-specific than sequence-specific, introduce slight conformational changes in the DNA structure thereby facilitating the subsequent interaction of specific transcription factors like NFκB and IRF3. Further recruitment of p300/CBP and the general transcription factors enables then the assembly of a highly stereospecific complex as it is described in the case of the 'Enhanceosome' for the transcription of the IFN-β gene. Furthermore, HMGA proteins can interact also directly with transcriptions factors like NFκB, p53 or pRB and influence their activity. Further target genes of HMGA proteins are described including Cyclins, Interleukins and metalloproteases.

HMGA proteins are abundant in undifferentiated und proliferating cells (like stem cells) whereas they are rather undetectable in fully differentiated cells suggesting a role in the differentiation process. However, misexpression of HMGA proteins in the development or in adult cells was shown to be involved in neoplastic transformation and tumor formation, a fact that is most likely directly connected to the large number of target genes of HMGA proteins and interactions to transcriptions factors. Besides skin, mammary, and lung carcinoma misexpression of HMGA proteins was also shown in B-cell lymphomas and myeloid leukemia's. However, there are two splice variants called HMGA1a and HMGA1b with different biochemical properties and the expression patterns of these are mostly unknown in these tumors. Furthermore, the HMGA proteins are target of a variety of posttranslational modifications like phosphorylation or acetylation. These modifications were previously shown to modulate the binding activity of the chromatin proteins, thereby facilitating a fine tuning of their function. Our aim is it to characterize tumor-specific signatures of the posttranslational modifications for different target genes of the HMGA proteins. Furthermore, HMGA proteins are important during early embryonic development. Using embryoid bodies cultures and siRNA-mediated knock-down we try to elucidated the role of HMGA proteins in mesodermal differentiation.

D Publications 2008/2009

Publications 2008	Impact Factor
1. Schwanbeck, R., Schroeder, T., Henning, K., Kohlhof, H., Rieber, N., Erfurth, M.L., Just, U. (2008) Notch Signaling in Embryonic and Adult Myelopoiesis <i>Cells Tissues Organs</i> 188: 91-102.	2.376
2. Henning, K.*, Heering, J.*, Schwanbeck, R.*, Schroeder, T.*, Helmbold, H., Schafer, H., Deppert, W., Kim, E., Just, U. (2008) Notch1 activation reduces proliferation in the multipotent hematopoietic progenitor cell line FDCP-mix through a p53-dependent pathway but Notch1 effects on myeloid and erythroid differentiation are independent of p53. <i>Cell Death Differ.</i> 15, 398-407.	7.548

*) denotes equal contribution

Impact factors 2008: 9.924

Impact factors 2009: -

Total impact factors 2008/2009: 9.924

E Grants

- E.1. Hensel-Stiftung, Analyse der architektonischen High-Mobility-Group A (HMGA) Chromatinproteine bei hochmalignen lymphatischen Neoplasien der B-Zell-Reihe. Fördersumme (XX) 48500 €.

13. Research Group Prof. Dr. Roland Schauer

A Group Leader: Prof. (em.) Dr. med. Dipl.-Biochem. Roland Schauer



B Lab Members:

Doctoral Students:

Vinayaga Srinivasan Gnanapragassam
Swantje Mindorf

C Research Report

C.1. The significance of sialic acids and the study of their *O*-acetylation

Sialic acids are gaining much interest in cell biology, from fertilization to cell death, and they are indispensable for maintaining the life of an eukaryotic cell of the deuterostome lineage. They were also found in some lower animals and are expressed e.g. in *Drosophila* and cicada during a short period of development. Sialic acids also occur in many microorganisms, mainly in bacteria and protozoa where they represent strong virulence factors.

Sialic acids mostly occupy the terminal positions of glycoproteins and glycolipids in monomeric or polymeric (polysialic acids) form on cell surfaces and thus are involved in the control of many cell functions, such as cellular interactions, transport and signalling events, differentiation, innate and acquired immunity, proteolysis, phagocytosis, apoptosis, and control of the life-time of macromolecules and cells. They also appear to have antioxidative effects. Generally, sialic acids have a masking, antirecognition effect on receptors and antigens, thus hindering for example cell recognition, autoimmune reactions or the removal of macromolecules and cells from the blood stream. On the other hand, they represent recognition sites, ligands, for sialic acids-binding proteins (lectins). Prominent examples for the latter are the siglecs in mammalian cells, mainly involved in erythropoiesis and immunoregulation, and the hemagglutinins of viruses. The binding of e.g. influenza viruses to sialic acids of erythrocytes and their release by virus neuraminidase led to the discovery of sialic acids and neuraminidase (sialidase) over 60 years ago. The binding of influenza viruses to a mammalian cell depends on the type of sialic acid glycosidic linkage to the penultimate sugar of glycan chains on cell surfaces. This strongly influences the species specificity of virus infection. Presently, many other virus species, such as corona- and rotaviruses, are being recognized to adhere to sialic acids in the process of cell infection. These monosaccharides are also involved in other pathological processes like cancer, bacterial and protozoal infections, e.g. malaria, sleeping sickness, *Helicobacter pylori* infection, and autoimmune diseases. Sialic acid derivatives were prepared which are potent inhibitors of virus propagation thus curing, for example, influenza (flu). A rapidly expanding area concerns the involvement of sialic acid in tumors, inflammation and neurodegenerative diseases.

There are more than 50 types of sialic acids known in nature with different *N*- and *O*-substituents (Fig. 1) as well as dehydro, anhydro and lactone forms. All are formally derived from *N*-acetylneuraminic acid (Neu5Ac). The biosynthesis of sialic acids, their subcellular sites and some of the modification reactions are shown in Fig. 2.

These chemical modifications influence the biology of cells, although only *N*-glycolylneuraminic acid (Neu5Gc) and *O*-acetylated derivatives of Neu5Ac like *N*-acetyl-4-*O*-acetylneuraminic acid (Neu4,5Ac₂) and *N*-acetyl-9-*O*-acetylneuraminic acid (Neu5,9Ac₂) have been studied in detail in this respect. They represent onco-fetal antigens and evoked much interest in tumor biology. Furthermore, Neu5Ac and Neu5,9Ac₂ were found to be specific receptors for many pathogenic microorganisms.

The Sialic Acid Family

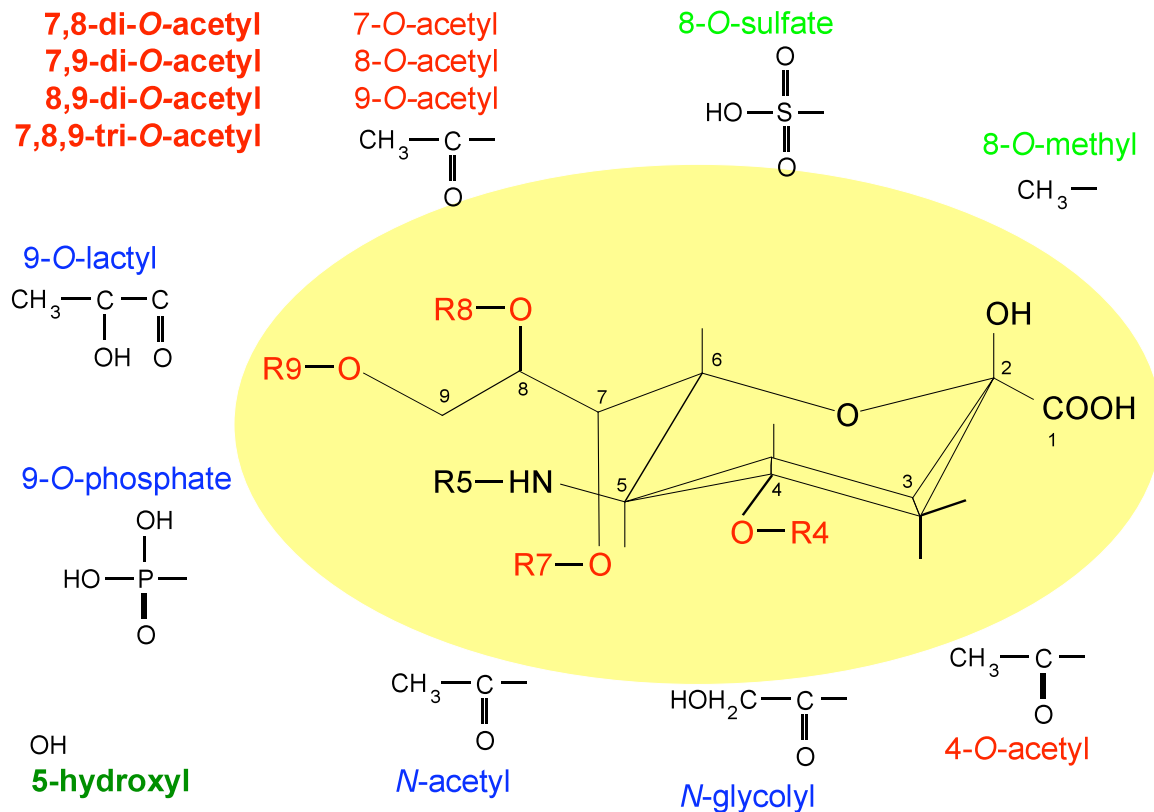


Figure 1: The sialic acid family

Our main interest focuses on the enzymology and molecular genetics of the *O*-acetylation of sialic acids. Due to the great (patho-)physiological significance of this sialic acid modification, knowledge of the regulation of the expression of the corresponding AcCoA:sialate-*O*-acetyltransferase(s) and its distribution in human tissues and animal species is most important. Neither was a eukaryotic *O*-acetyltransferase isolated nor cloned or expressed so far, in spite of many attempts made in various laboratories.

Studies using a novel and faster enzyme test and a modified enzyme protein isolation procedure have shown that the enzyme is part of a protein complex in the Golgi membrane, composed by transporters for AcCoA, CMP-sialic acids, the *O*-acetyltransferase and perhaps also sialyltransferase (Fig. 3). Since the primary insertion site of the *O*-acetyl group was found to be at C-7 of the sialic acid side chain, the existence of an isomerase ("migrase") necessary for the distribution of *O*-acetyl groups from C-7 to C-8 and C-9 is still discussed. Evidence for the involvement of an activator in the *O*-acetylation process was obtained. Sialate-*O*-acetyltransferase is high in human lymphocytes and especially active in acute lymphoblastic leukemia (ALL) lymphocytes. On remission of the disease, enzyme activity declines. Esterases specifically saponifying *O*-acetylated sialic acids are investigated as well.

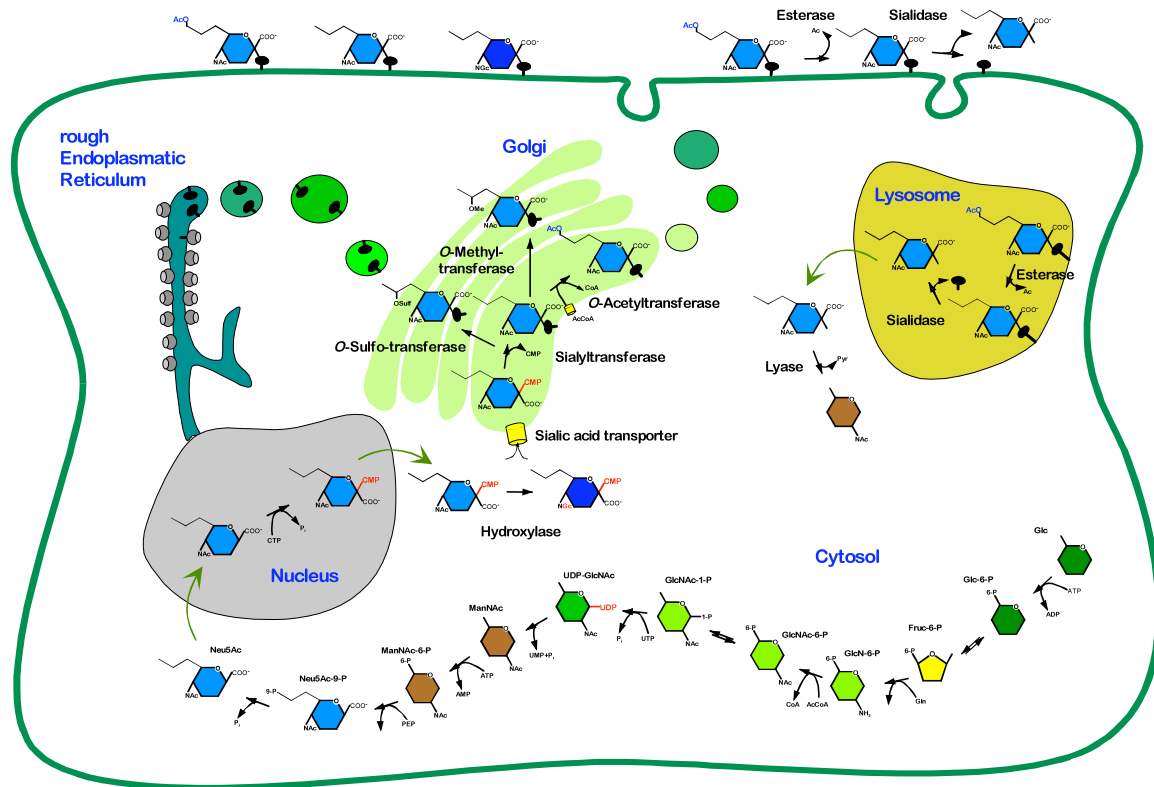


Figure 2: Metabolism of sialic acids

We are studying the *O*-acetylation of sialic acids in cooperation with Dr. Chitra Mandal (Kolkata) in acute lymphoblastic leukemia (ALL) and with Dr. Reinhard Schwartz-Albiez (Heidelberg) in human B- and T-lymphocytes. With Prof. Reinhard Vlasak (Salzburg) a gene is under investigation which is conserved from the fungus *Cryptococcus neoformans* to man. It seems to code for a Golgi-bound acetyltransferase and is suspected to be responsible for expression of the enigmatic sialic acid enzyme.

C.2. Occurrence of *N*-glycolylneuraminic acid

N-Acetyl hydroxylation yielding Neu5Gc is, like *O*-acetylation, a frequent sialic acid-modifying reaction. Interest in this substance is increasing, since it was found that man does not express Neu5Gc, due to a gene mutation which occurred in evolution during the human divergence from great apes. This is of practical significance in microbial and non-microbial inflammation and one of the reasons why we are interested in the distribution of this monosaccharide in nature. The other reason are evolutionary aspects and we are focussing on sauropsids, such as birds and reptiles, and on monotremes like the platypus and echidna. The tools for these studies are chemical and molecular genetic analyses. A cooperation exists with Dr. Yann Guérardel (Lille) and Prof. Michael Messer (Sydney). We have found that tissues of the many bird and reptile species we studied do not express Neu5Gc, with the exception of a few eggs of these animals. The Australian monotremes, like the platypus, do also not possess the gene for Neu5Gc synthesis, the CMP-Neu5Ac hydroxylase (*cmah*). These observations may be of nutritional significance for humans: Since man has lost *cmah*, Neu5Gc ingested from food, e.g. from red meat, is a xeno-antigen which is discussed to cause inflammation e.g. in intestinal mucosa leading to cancer.

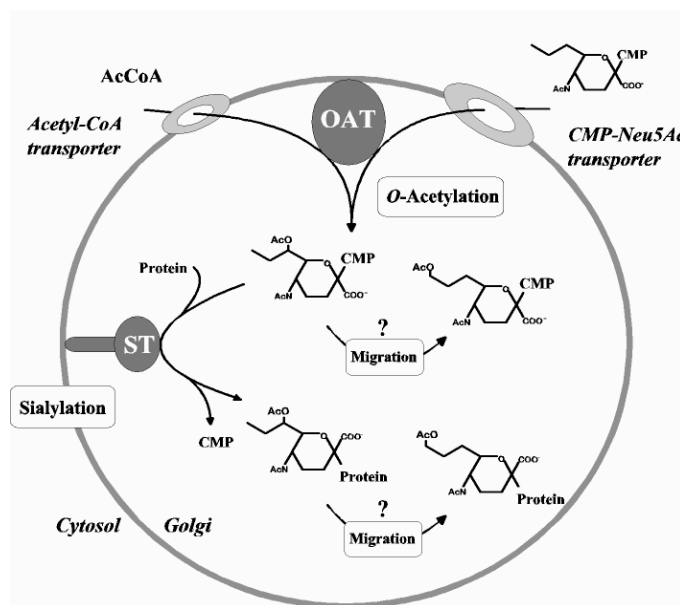


Figure 3: The postulated pathway for the *O*-acetylation of sialic acids

C.3. Trypanosomal Trans-Sialidases

These enzymes were found in pathogenic trypanosomes such as the African *Trypanosoma brucei* and *T. congolense*, and the American *T. cruzi*, leading to sleeping sickness and Chagas Disease, respectively. Trans-Sialidases can act as sialidases or as sialyltransferases. In the case of the absence of suitable glycosyl acceptors, trans-sialidases hydrolytically release sialic acids from their glycosidic bonds such as classical sialidases (neuraminidases). They, however, prefer to transfer sialic acids from α 2,3-glycosidic bonds of glycans to terminal galactose residues of other glycoconjugate molecules yielding new α 2,3-glycosidic linkages. In this way trypanosomes acquire sialic acids from the host and thus increase their virulence, especially by compromising the host's immune system.

Therefore, inhibitors of trypanosomal trans-sialidases are expected to be suitable for therapy of these frequent and disastrous tropical diseases. We are testing, together with Dr. Silke Schrader (Köln), inhibitors of *T. cruzi* trans-sialidase synthesized by Prof. J. Thiem (Hamburg) and Prof. Teruo Yoshino (Tokyo).

D Publications 2006/2007

Publications 2008	Impact Factor
1. R. Schauer and A.K. Shukla (2008) Isolation and properties of two sialate- <i>O</i> -acetyltransferases from horse liver with 4- and 9- <i>O</i> -acetyl specificities. <i>Glycoconjugate J.</i> 25,625-632.	1.743
Publikationen 2009	Impact Factor
1. C. Mandal, G.V. Srinivasan, S. Chowdhury, S. Chandra, C. Mandal, R. Schauer, Mandal (2009) High level of sialate- <i>O</i> -acetyltransferase activity in lymphoblasts of childhood acute lymphoblastic leukaemia (ALL): enzyme characterization and correlation with disease status. <i>Glycoconjugate J.</i> 26:57-73.	1.743
2. Srinivasan, GV and Schauer, R. (2009) Assays of sialate <i>O</i> -acetyltransferases and sialate- <i>O</i> -acetyltransferases. <i>Glycoconjugate J.</i> 26: 936-944.	1.743
3. Siebert, HC, Lu, SY, Wechselberger, R, Born, K, Eckert, T, Liang, S, von der Lieth, CW, Jiménez-Barbero, J, Schauer, R, Vliegenthart, JFG, Lütke, T, Kočár T.(2009). A lectin from the Chinese bird-hunting spider binds sialic acids. <i>Carbohydr. Res.</i> 344,1515-1525.	1.960

4. Schauer, R. (2009) Sialic acids as regulators of molecular and cellular interactions. <i>Curr. Opin. Struct. Biology</i> , 19:507-514.	9.060
5. Varki, A, Schauer, R. (2009) Sialic Acids, (chapter 14) IN <i>Essentials of Glycobiology</i> . 2 nd edition. Varki, A., Cummings, R.D., Esko, J.D., Freeze, H.H., Stanley, P., Bertozzi, C.R., Hart, G.W., and Etzler, M.E., eds., pp. 199-217, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.	
6. Schauer, R, Srinivasan, GV, Coddeville, B, Zanetta, JP, Guérardel, Y. (2009) Low incidence of <i>N</i> -glycolylneuraminic acid in birds and reptiles and its absence in the platypus. <i>Carbohydr. Res.</i> 344:1494-1500.	1.960

Impact factors 2008: 1.743

Impact factors 2009: 16.466

Total impact factors 2008/2009: 18.209

E Grants

Currently no grants

F Awards

2009 Rosalind Kornfeld Award for Lifetime Achievement in Glycobiology jointly awarded to Mary Catherine Glick, Pennsylvania, and Roland Schauer, Kiel by the Society for Glycobiology, San Diego (USA) *Glycobiology* (2009) 19:1152–1154

Appendix

Seminars 2008/2009

15. Jan. 2008 **Prof. Dr. Thomas Blankenstein, MDC Berlin**
The immune response to sporadic immunogenic cancer
29. Jan. 2008 **Prof. Dr. Axel Scheidig, ZBM, CAU Kiel**
Gaining insight into the mode of action of enzymes using x-ray crystallography - a case study on DNA-methyltransferase, PAPS-synthase 1 and protein tyrosine phosphatase.
05. Feb. 2008 **Prof. Dr. Bruno Gasnier, Université Denis Diderot, Paris**
Lysosomal membrane transporters
22. April 2008 **Prof. Dr. Norbert Tautz, Universität Lübeck**
Control of viral protease activity - at the crossroads between virus persistence and lethal disease
06. Mai 2008 **Prof. Dr. Eberhard Hildt, Mikrobiologie, UKSH Kiel**
Hepatitis B and Hepatitis C Virus: viral entry and replication strategies
27. Mai 2008 **PD Dr. Regina Fluhrer, LMU München**
Presenilins and SPP/SPPLs – Two GxGD-type Aspartylprotease Families, Similarities and Differences
03. Juni 2008 **Dr. Hendrik Oster, MPI Biophysikalische Chemie, Göttingen**
Clock Genes in Endocrine Regulation
17. Juni 2008 **Prof. Dr. Maria Sibilina, Universität Wien**
The epidermal growth factor receptor: from development to tumorigenesis
01. Juli 2008 **Dr. Frank Sönnichsen, Christian-Albrechts-Universität Kiel**
NMR Spectroscopy on Membrane Proteins.
08. Juli 2008 **Prof. Hans Aerts, Academic Medical Center, Amsterdam**
Glycosphingolipids and insulin resistance (Type 2 diabetes)
04. Nov. 2008 **Prof. Harald Kolmar, Technische Universität Darmstadt**
Alternative binding proteins: biological activity and therapeutic potential of cystine-knot miniproteins
18. Nov. 2008 **Dr. Catherine Meyer-Schwesinger, UKE Hamburg**
Protein Degradation pathways in podocyte injury
09. Dez. 2008 **Dr. Kay Grobe, Universität Münster**
ADAM-mediated release of Sonic hedgehog (Shh) from producing cells
12. Dez. 2008 **Prof. Dr. Volker Haucke, Freie Universität Berlin, Germany**
Regulation of recycling endosomal membrane traffic by lipid metabolizing enzymes and microtubular motor proteins
15. Jan. 2009 **Prof. Gillian Murphy, Dept of Oncology, University of Cambridge, UK**
Regulation and Inhibition of ADAM Proteases
03. Feb. 2009 **Prof. Dr. Ulrich Schaible, Forschungszentrum Borstel**
Intimate host-pathogen hobnobbing in tuberculosis.
30. April 2009 **Dr. Björn Schröder, Max-Delbrück-Center, Berlin**

TMEM16A: Expression cloning of a new family of calcium activated chloride channels.

19. Mai 2009 **PD Dr. Heike Hermanns, Virchow Zentrum, Universität Würzburg**
Immunoregulatory functions of the interleukin-6-type cytokine Oncostatin M
02. Juni 2009 **PD Dr. Matthias Wilmanns, EMBL Hamburg**
Molecular basis of homo/hetero-assembly in bZIP transcription factors
09. Juni 2009 **Prof. Dr. Michael Martin, Institut für Immunologie, Universität Giessen**
Interleukin-33 and its receptor complex
12. Juni 2009 **Prof. Dr. Nils Brose, MPI Experimentelle Medizin Göttingen**
Regulation of membrane fusion in synaptic excitation-secretion coupling: speed and accuracy matter.
30. Juni 2009 **Prof. Dr. Renate Kain, Medizinische Universität Wien**
Mechanisms of autoimmunity to LAMP-2: a case for molecular mimicry
07. Juli 2009 **PD Dr. Florian R. Greten, Medizinische Klinik, Technische Universität München**
Molecular mechanisms linking inflammation and intestinal tumorigenesis
01. Dezember 2009 **Prof. Dr. Fred Schaper, Aachen**
New concepts for the regulation of interleukin-6 type cytokine signalling
08. Dezember 2009 **Dr. Dirk Schmidt-Arras, Paris**
Kinase Signaling in Leishmania Virulence and Leukemia
15. Dezember 2009 **Prof. Dr. Tamas Laskay, Lübeck**
Phagocytosis of apoptotic cells by neutrophils

Publications 2008/2009

Original Papers and Reviews

- Adam N, Rabe B, Suthaus J, Grötzinger J, Rose-John S and Scheller J (2009) Understanding receptor independent gp130 activation of viral interleukin-6. *J Virol* 83: 5117-5126 5.308
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Accumulated Impact Factors

2002 = 220.15

2003 = 327.63

2004 = 320.06

2005 = 318.73

2006 = 380.806

2007 = 258.401

2008 = 292.875

2009 = 311.386

