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Additional Members of Thesis Advisory Committees: R. Marienfeld (Ulm), R. Wetzker (Jena), T. Böckers (Ulm), T. Wieland (Mannheim), K.D. Spindler (Ulm), M. Thelen (Bellinzona), S. Kochanek (Ulm), M. Schwarz (Tübingen), C. Montecucco (Padua), G. Fischer (Halle)

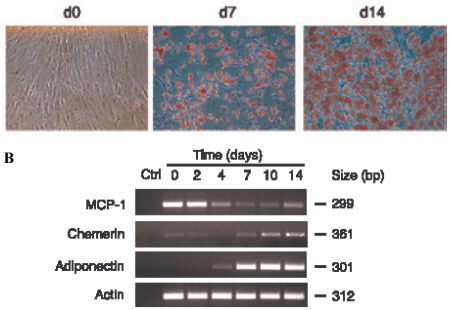
Institute of Pharmacology and Toxicology

Signal Transduction Mediated by Heterotrimeric and Rho GTPases

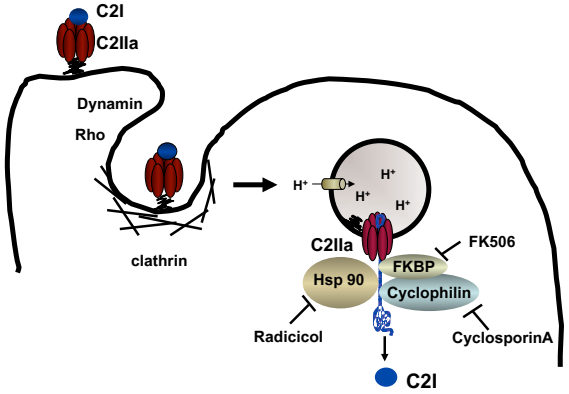
Head: Peter Gierschik

GTPases bind and hydrolyze GTP and are members of a large family of proteins involved in collecting, integrating, processing and distributing extracellular and intracellular information to regulate and orchestrate many fundamental aspects of cell function, such as cell proliferation, migration, differentiation and apoptosis, as well as multiple specialized cell functions, including secretion, contraction, phagocytosis and various sensory and neuronal as well as immune cell functions. Aside from their central role in cell biology and physiology, GTPases are of paramount clinical importance for contributing to the pathogenesis of human diseases and to the action of a major portion of the drugs currently used in clinical practice.

Three doctoral projects pursued at the institute are concerned with the structure-function relationships of GTPases, two of which have been completed recently. Mariana Pfreimer investigated the mode of Rho GTPase activation by chemokine receptors through heterotrimeric GTPases (G proteins) and the **l**eukemia-**a**ssociated **R**ho **g**uanine nucleotide exchange factor (RhoGEF) LARG. Carolin König explored the impact of G-protein-coupled receptors on terminal differentiation and trafficking of human adipocytes and/or their interaction with monocytes/macrophages and other cell types in the adipose tissue. Anja Bühler studies the mechanisms of phospholipase C-γ_2 (PLCγ_2) activation by the Rho GTPase Rac2 and the molecular mechanisms of PLCγ_2 activation in mutants found in human hereditary autoinflammatory diseases and in animal models of such disorders.



Expression of chemokines and adipokines in human adipose lineage cells during terminal adipocyte differentiation. *A*, Simpson-Golabi-Behmel (SGBS) preadipocytes, cultured cells capable of differentiating *in vitro* into mature white adipocytes were subjected to a 14 day differentiation process, which was controlled by Oil Red O staining of the cellular lipid content. Cells of day 0 *(d0)*, day 7 *(d7)*, and day 14 *(d14)* are shown. *B*, Reverse transcriptase polymerase chain reaction expression analysis of mRNAs encoding the human CC chemokine monocyte chemoattractant protein 1 (MCP-1) or the adipokines chemerin and adiponectin in SGBS cells at days 0 through 14 of the differentiation protocol. *Ctrl*, control sample without single-stranded template cDNA. Adapted from Koenig et al. (2013) Mol. Cell. Endocrinol. 369:72.



Role of host cell chaperones in uptake of the binary C2 toxin from *Clostridium botulinum*. (modified from Barth H, Aktories K (2011) E J Cell Biol 90:944-950)

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Cellular Uptake of Bacterial Exotoxins and Their Use as Molecular Trojan Horses for Drug Delivery

Bacterial exotoxins are proteins which enter mammalian cells and enzymatically modify specific substrates in their cytosol. This results in cell damage and the symptoms of severe diseases such as diphtheria, anthrax or botulism. For cell entry, a binding/translocation domain mediates the transport of an enzyme domain into the cytosol. This unique mode of action makes exotoxins ideal transporters for targeted delivery of macromolecules into cells. We investigate the molecular mechanisms underlying the toxin transport into target cells and develop tailor-made transporters based on enzymatic inactive toxin fragments thereof to deliver pharmacologically active molecules into the cytosol of monocytes/macrophages.

Maren Lillich and Hannes Christow constructed toxin-based transporters to deliver mammalian proteins into cells by coupling the binding/transclocation moieties of clostridial toxins to streptavidin, either by genetic fusion and expression of the recombinant protein or by chemical crosslinking in vitro. Biotin-labeled molecules bound to the streptavidin domain are delivered by the fusion toxins into various cell types including tumor cells. Katharina Ernst studies the role of host cell chaperones in mediating the uptake of bacterial toxins into the host cell cytosol. She demonstrated that various peptidyl prolyl *cis/trans* isomerases specifically facilitate the intracellular membrane transport of ADP-ribosylating toxins by directly interacting with their catalytic domains. Lydia Dmochwitz develops and characterizes recombinant fusion toxins based on an enzymatically inactive variant of C3 toxin from *Clostridium botulinum*, which is selectively taken up into monocytes/macrophages. Since C3-based transporters serve the selective delivery of enzymes into cultured monocytes/macrophages, the vision behind this approach is their therapeutic use in monocyte/macrophage-associated diseases.

Selected Publications:

- *Pfreimer M, Vatter P, Langer T, Wieland T, Gierschik P, Moepps B (2012): LARG links histamine-H1-receptor-activated Gq to Rho-GTPase-dependent signaling pathways. Cell. Signal. 24:652-663.*
- *Koenig C, Fischer-Posovszky P, Rojewski MT, Tews D, Schrezenmeier H, Wabitsch M, Gierschik P, Moepps B (2013): Absence of CC chemokine receptors 2a and 2b from human adipose lineage cells. Mol. Cell. Endocrinol. 369:72-85.*
- *Lillich M, Chen X, Weil T, Barth H, Fahrer J (2012): Streptavidin-conjugated C3 protein mediates the delivery of mono-biotinylated RNAse A into macrophages. Bioconjug. Chem. 23: 1426-1436.*
- *Christow H, Lillich M, Sold A, Fahrer J, Barth H (2013): Recombinant streptavidin-C3bot for delivery of proteins into macrophages. Toxicon, in press.*
- *Dmochewitz L, Förtsch C, Zwerger C, Väth M, Felder E, Huber-Lang M, Barth H (2013): A recombinant fusion toxin based on enzymatic inactive C3bot1 selectively targets macrophages. PLoS One 8:e54517.*
- *Kaiser E*, Böhm N*, Ernst K*, Langer S, Schwan C, Aktories K, Popoff MR, Fischer G, Barth H (2012): FK506-binding protein 51 interacts with Clostridium botulinum C2 toxin and FK506 blocks membrane translocation of the toxin in mammalian cells. Cell. Microbiol. 14:1193-1205. (*contributed equally)*