Focus of the PhD-Project (Prof. Dr. Jens Rettig)

Molecular mechanisms of lytic granule release at the immunological synapse

Upon activation, CD8-positive T-lymphocytes form a spezialized contact site with antigen-presenting cells, the immunological synapse (IS). At the IS the T-lymphocytes release perforin and different granzyme isoforms through fusion of lytic granules with the plasma membrane. Subsequently the antigen-presenting cell is being killed through lysis mediated by perforin and granzymes. A number of mostly lethal diseases (e.g. familial hemophagocytic lymphohistiocytosis) are caused by mutations in involved proteins and underscore the importance of the process.

In a close collaboration with the group of Markus Hoth (Biophysics) we are investigating the molecular machinery that drives this process and eventually enables the fusion of lytic granules. We label lytic granules with fluorescent marker proteins and follow their transport towards the IS in real-time with the high resolution-methods described below. At the IS we employ TIRF microscopy in combination with molecular manipulations to quantify membrane-bound processes such as granule docking and priming in wild-type and mutant T-lymphocytes. We are focussing our attention on SNARE ("soluble N-ethylmaleimide-sensitive factor attachment protein receptor") proteins that are well-established mediators of Ca^{2+}-dependent vesicle fusion in neurons and neuroendocrine cells. For the processes preceding the fusion event we concentrate on proteins which might interact with SNARE proteins and modulate their assembly. For these purposes we have a number of transgenic animals carrying deletions of specific genes at our disposal.

Methods:
Total internal reflection fluorescence microscopy; confocal microscopy; Ca^{2+} imaging; Generation and breeding of knockout- and knockin-mice; Isolation, transfection and culture of human and mouse T cells; Molecular biology and many more.

Selected publications:

*Yizhar, O., *Matti, U., Melamed, R., Hagallili, Y., Bruns, D., #Rettig, J. and #Ashery, U. (2004). Tomosyn regulates exocytosis in a calcium-dependent manner. *Proc. Natl. Acad. Sci. USA* **101**, 2578-2583.

Nagy, G., Reim, K., Matti, U., Brose, N., Binz, T., Rettig, J., Neher, E. and Soerensen, J. (2004). Regulation of releasable pool sizes by protein kinase A-dependent phosphorylation of SNAP-25. *Neuron* **41**, 351-365.

*Thakur, P., *Stevens, D.R., Sheng, Z.-H. and Rettig, J. (2004). Effects of PKA-mediated phosphorylation of snapin on synaptic transmission in cultured hippocampal neurons. *J. Neurosci.* **24**, 6476-6481.

*Speidel, D., *Bruederle, C.E., Enk, C., Voets, T., Varoqueaux, F., Reim, K., Becherer, U., Fornai, F., Holighaus, Y., Weihe, E., Bruns, D., #Brose, N. and #Rettig, J. (2005). CAPS1 regulates catecholamine loading of large dense-core vesicles. *Neuron* **46**, 75-88.

*Tian, J.H., *Wu, Z.X., Unzicker, M., Li, L., Lu, L., Schirra, C., Matti, U., Stevens, D., Deng, C., #Rettig, J. and #Sheng, Z.-H. (2005). A Role of Snapin in Neurosecretion: Snapin Knockout Mice Exhibit Impaired Calcium-dependent Exocytosis of Large-dense Core Vesicles in Chromaffin Cells. *J. Neurosci.* **25**, 10546-10555. **IF: 8.4; TC: 12**

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*Nofal, S., *Becherer, U., Hof, D., Matti, U. and Rettig, J. (2007). Primed vesicles can be distinguished from docked vesicles by analyzing their mobility. *J. Neurosci.* **27**, 1386-1395.

*Becherer, U., *Pasche, M., *Nofal, S., Hof, D., Matti, U. and Rettig, J. (2007). Quantifying exocytosis by combination of membrane capacitance measurements and total internal reflection fluorescence microscopy. PloS ONE 2, e505.

Quintana, A., Schwindling, C., Wenning, A.S., Becherer, U., Rettig, J., Schwarz, E.C. and Hoth, M. (2007). T cell activation requires mitochondrial translocation to the immunological synapse. *Proc. Natl. Acad. Sci. USA* **104**, 14418-14423.

Liu, Y., Schirra, C., Stevens, D.R., Matti, U., Speidel, D., Hof, D., Bruns, D., Brose, N. and Rettig, J. (2008). CAPS facilitates filling of the rapidly releasable pool of large dense core vesicles. *J. Neurosci.* **28**, 5594-5601.

Zeniou-Meyer, M., Liu, Y., Béglé, A., Olanish, M., Hanauer, A., Becherer, U., Rettig, J., Bader, M.F. and Vitale, N. (2008). The Coffin-Lowry syndrome-associated protein RSK2 is implicated in calcium-regulated exocytosis through the regulation of PLD1. *Proc. Natl. Acad. Sci. USA* **105**, 8434-8439.