

Seminars
Frontiers of Biology of Cell Systems
(Funded by FNR)

Thursday, 17th December 2009 at 15h00

Campus Limpertsberg

Bâtiment des Sciences 2.01

Dr. Rainer Pepperkok, EMBL Heidelberg will speak about

"Illuminating the secretory pathway"

We have developed an organelle knock-out approach in which we remove by laser nano-surgery the entire Golgi complex from living cells and subsequently follow by time-lapse and electron microscopy analysis the "Golgi-less" karyoplast. The data obtained strongly support the hypothesis of a *de novo* Golgi biosynthesis. To identify putative molecules involved in this *de novo* Golgi biogenesis, we have developed and applied functional assays to assess the effect of knock-ins by cDNA over-expression and knock-downs by RNAi, on processes such as constitutive protein transport, Golgi integrity and function of vesicular coat complexes. To achieve the throughput that such analyses require we have developed a fully automated high content screening microscopy platform including sample preparation, image acquisition and automated analysis of complex cellular phenotypes. We have applied this technology to genome-wide siRNA screens to identify and characterize comprehensively the genes and their underlying functional networks involved in secretory membrane traffic and Golgi integrity.

References:

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- Erfe, et al. 2007. Reverse transfection on cell arrays for high content screening microscopy. *Nat Protoc.* 2:392-9.
- Simpson et al 2007. An RNAi screening platform to identify secretion machinery in mammalian cells. *J Biotechnol.* 129:352-65.
- Pepperkok, R. and J., Ellenberg. 2006. High-throughput fluorescence microscopy for systems biology. *J. Nat Rev Mol Cell Biol.* 7:690-6
- Neumann et al. 2006. High-throughput RNAi screening by time-lapse imaging of live human cells. *Nat. Methods* 3: 385-390.