Room O1 LM

Endocytosis: from single molecules to organism development

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Exocytosis and endocytosis; from membranes and molecules to mechanisms

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Cell shape is adapted to function. Organelle shape and local membrane architectures are likewise optimised for the processes that take place on and within these microenvironments. We focus on the dynamic regulation of membrane shape, which can occur by the interplay between the transient and regulated insertion of membrane bending motifs and the detection and stabilisation of membrane shape. This approach has allowed us not only to describe the biophysics of membrane shape changes but also to take a fresh look at membrane dynamics in physiological processes like exocytosis and endocytosis. In doing so we have noted that proteins with amphipathic helices or hydrophobic membrane-inserting loops are likely to effect or respond to curvature and that the membrane interaction surfaces of proteins can sense shape (like proteins of the BAR Superfamily). This molecular view has allowed us to ascribe novel cell-biological functions to proteins (e.g. the mechanistic affect of synaptotagmin in membrane fusion) and to give a more insightful view of how these processes work. Thus we can now go from the biophysics of a molecule, to better understanding of known pathways and to the molecular characterisation of novel cellular trafficking pathways both of endocytosis and exocytosis. See: http://www.endocytosis.org/

Coupling the membrane to the actin cytoskeleton during endocytosis

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Local actin polymerization is thought to provide force for the membrane shape changes that take place during clathrin-mediated endocytosis. However, the molecular mechanism by which the force from the actin cytoskeleton is transmitted to the membrane has been unknown. We show that in budding yeast, *Saccharomyces cerevisiae,* Ent1 (epsin) and Sla2 (homolog of mammalian HIP1R) proteins couple the membrane to the actin cytoskeleton during endocytic vesicle formation. Ent1 and Sla2 interact with the membrane cooperatively through their N-terminal lipid-binding domains. Furthermore, Sla2 and Ent1 bind in a redundant manner to the actin filaments. The interaction between Ent1 and the actin cytoskeleton is mediated by a novel actin-binding domain, which is regulated by phosphorylation. By their synergistic binding to the membrane and redundant interaction with the actin cytoskeleton, Ent1 and Sla2 form a unique molecular linker that transmits the force generated by the actin cytoskeleton to the invaginating membrane.

**Oral presentation selected from abstracts

Systems analysis of endocytosis

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Endocytosis is an essential process serving multiple key cellular functions, such as nutrient uptake, signal transduction, and defence against pathogens. We have undertaken a broad systems biology analysis of endocytosis. We systematically profiled the activity of human genes with respect to Transferrin and EGF endocytosis by performing an image-based RNAi screening of HeLa cells in cooperation with the HT-TDS, the screening facility of the MPI-CBG. The genes were identified on the basis of a multi-parametric analysis quantitatively measuring uptake and intracellular cargo distribution. We uncovered novel regulators of endocytosis and endosome trafficking, including many signalling pathways (e.g. Wnt, Integrin, TGF- , and Notch). A systems analysis by Bayesian networks further uncovered design principles regulating the number, size, concentration of cargo and intracellular position of endosomes. Based on these results we formulated and tested the hypothesis that the endosomal distribution of signalling molecules may regulate the extent and duration of the signal output. Our results reveal novel principles whereby the endocytic pathway governs the sorting and signalling properties of receptor tyrosine kinases.

Membrane scaffolds in endocytosis – from molecules to systems

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The maintenance of synaptic transmission depends on the biogenesis and recycling of presynaptic vesicles (SVs), specialized organelles that store and secrete non-peptide neurotransmitters. How the full complement of SV proteins is maintained during multiple rounds of exo-endocytosis is unknown. While some data suggest a SV clustering-based mechanism other results argue in favor of a model whereby SV proteins dispersed at the axonal surface are sorted individually, presumably in a cargo- and adaptor-specific fashion. In my talk I will focus on the general role of clathrin as an endocytic scaffold in endocytosis and SV recycling. Furthermore, I will address how SV proteins are recognized and sorted by cargo-specific adaptors at synapses.

Multivariate clathrin-coated vesicle profiling

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Clathrin-coated vesicles (CCVs) are major endocytic and intracellular transport intermediates. Here we report a multivariate comparative proteomics approach to mapping the machinery and cargo of CCVs in HeLa cells. We establish multiple objective criteria that allow us to distinguish CCV proteins from co-purifying contaminants, and also to differentiate between different types of CCVs.

We used SILAC-based quantitative mass spectrometry to compare the composition of CCV fractions prepared under different experimental conditions. Over 2,500 proteins were identified in the CCV fraction. Cluster delineation based on Principal Component Analysis predicts that 133 of these proteins are CCV associated, including 36 novel proteins. Furthermore, the clustering also predicts if a CCV protein functions in endocytosis or intracellular trafficking. Our analysis identified >91% of known CCV proteins, and assigned >93% of them correctly as

either intracellular or endocytic. Therefore, the novel predicted CCV proteins can be functionally assigned with high confidence. Although the experimental design is optimized to identify CCV proteins, a by-product of the multivariate clustering is a comprehensive analysis of the >2300 'contaminant' proteins present in the CCV fraction. Numerous known and novel associations among non-CCV proteins were detected. These included for example 16 Retromer-associated proteins, five of which had not been reported previously. Six of the novel proteins were analysed by immunofluorescence microscopy, and all had the predicted sub-cellular localization.

This study is the most complete characterization of the CCV proteome to date. Although we focused on clathrin-mediated trafficking, we also uncovered many associations among non-CCV proteins. This powerful approach can be adapted to address related cell- and systems biological questions.

PICK1 is a novel interactor of NEPH/nephrin proteins regulating cell recognition and morphogenesis

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Diseases of the glomerular filter of the kidney are a leading cause of end-stage renal failure. A major constituent of this filter is the slit diaphragm, a highly specialized cell-cell contact of podocytes, the visceral epithelial cells of the glomerulus. The slit diaphragm plays a critical role for the size-selective filtration barrier of the kidney. The immunoglobulin superfamily proteins NEPH1-3 and nephrin contribute to the structure and the signalling capacity of the slit diaphragm.

Using yeast-two hybrid screens we identified the PDZ/BAR domain protein PICK1 as a novel interactor of NEPH/nephrin proteins. Here we provide further biochemical evidence for this interaction, as well as evidence for a role of PICK1 as regulator of the endocytosis of the NEPH/nephrin protein complex. NEPH/nephin proteins are evolutionarily highly conserved. In the fruit fly D. melanogaster as well as in the nematode *C. elegans* it has been shown, that this set of proteins is critically involved in developmental processes that require highly regulated cell-cell recognition and formation of cell-cell contacts. Among these processes in *Drosophila* is the development of the regular pattern of the fly eye. Our data demonstrate that RNAi mediated knock-down of PICK1 interferes with eye development in a way, that is highly reminiscent of the phenotype that occurs when interfering with NEPH/nephrin-like proteins. Thus, our findings suggest that NEPH/nephrin proteins and PICK1 are indeed involved in the same biological pathway.

We identified PICK1 as a novel interactor of NEPH/nephrin proteins. These proteins act together in eye development of the fly, a process that requires highly regulated cell-cell recognition and formation of cell-cell contacts. Further studies will have to clarify the role of PICK1 in the kidney.