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Cell Polarity and Membrane Traffic

Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava) • Spain
23 -28 May 2009

Chair: **Anne Spang,** Biozentrum University of Basel, CH
Co-Chair: **Ian G. Macara**, University of Virginia, US

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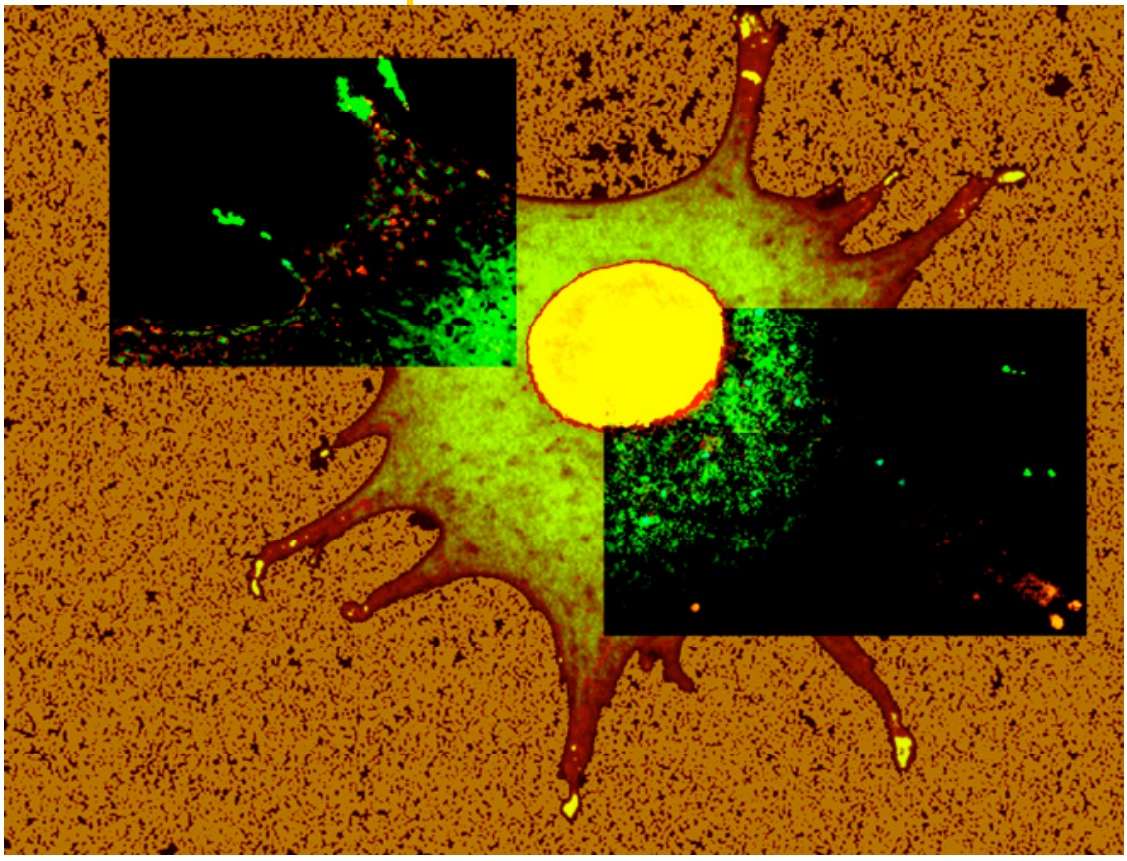

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Final Programme

Saturday 23 May

Late afternoon / early evening	Registration at the ESF-RC desk
19.00	Dinner
20.30	**Keynote Lecture : Yuh Nung Jan** HHMI, UCSF, US *An exuberant example of cell polarity: How do axons and dendrites acquire their distinct properties*
21.15	Welcome drink

Sunday 24 May

08.45-09.00	Conference Opening **Pilar Pérez** CSIC, ES *Presentation of ESF*

Session 1:

Chair: Claudia Stürmer, University of Konstanz, DE

09.00-09.35	**Kai Simons** Max-Planck-Institute of Molecular Cell Biology and Genetics, DE *Lipid rafts and membrane trafficking*
09.40-10.15	**Philippe Chavrier** Institut Curie - CNRS UMR144, FR *Mechanism of invadopodia formation by tumor cells*
10.20-10.40	Coffee break
10.45-11.20	**Catherine Rabouille** UMC Utrecht, NL *Unconventional secretion to the basal side of epithelial cells*
11.25-12.00	**Anne Spang** Biozentrum University of Basel, CH *SAND-1 is the critical switch for early-to-late endosome transition*
12.05-12.25	**George Banting – Short Talk** Bristol University, UK *A CD317(tetherin)/RICH2 complex plays a critical role in the organisation of the sub-apical actin cytoskeleton in polarised epithelial cells*
12.30	Lunch
15.00-16.00	Poster session and Coffee

Session 2:

Chair: Rytis Prekeris, University of Colorado Denver, US

16.00-16.35
Michel Labouesse
Université Louis Pasteur – IGBMC, FR
Junction remodelling during C. elegans embryonic morphogenesis

16.40-17.15
Pilar Perez
CSIC, ES
Fission yeast Cdc42 links cytoskeleton assembly and secretion to generate polarized growth

17.20-17.17.55
Patrick Brennwald
UNC, US
Rho GTPase Regulation of Cell Polarity and Exocytosis

18.00-18.20
Aniko Keller-Pinter – Short Talk
University of Szeged, HU
Syndecan-4 regulates the Rac1 activity and influences the localization of Par6 subsequently mediating epithelial-mesenchymal transition

18.25-18.45
Mary Munson - Short Talk
University of Massachusetts Medical School , US
Sec6p anchors the assembled exocyst complex at sites of secretion

19.00 Dinner

20.30-22.00 Poster Session: Drinks Sponsored by Roche

Monday 25 May

Session 3

Chair: Pierre Courtoy, University of Louvain Medical School, BE

09.00-09.35
Julie Ahringer - Biochemical J. lecture
University of Cambridge, UK
Cell Polarity in C. elegans embryos

09.40-10.15
David Bilder
University of California, Berkeley, US
Lecture

10.20-10.40 Coffee break

10.45-11.20
Daniel St. Johnston
University of Cambridge, UK
Lecture

11.25-12.00
Anne Ephrussi
European Molecular Biology Laboratory (EMBL), DE
Visualizing oskar RNP assembly for polarized transport in the Drosophila Oocyte

12.05-12.25
Elizabeth Gavis – Short Talk
Princeton University, US
Visualizing mRNA transport pathways

12.30 Lunch

15.00-16.00 Poster Session and Coffee

Session 4

Chair: Dirk Niessing, Helmholtz-Zentrum München, DE

16.00-16.35 **Ernst Stelzer**
European Molecular Biology Laboratory (EMBL), DE
Light sheet based fluorescence microscopes (LSFM, SPIM, DSLM) reduce phototoxic effects by several orders of magnitude

16.40-17.15 **Hans Meinhardt**
Max Planck Institute for Developmental Biology, DE
Models for the generation of highly dynamic intracellular patterns

17.20-17.55 **Matthias Weiss**
German Cancer Research Center (DKFZ), DE
Combining microscopy and simulations to elucidate generic aspects of membrane traffic

18.00-18.20 **Katrin Willig – Short Talk**
Max-Planck-Institute for Biophysical Chemistry , DE
STED nanoscopy in living cells using live cell compatible markers

18.25-18.45 **Stefanie Redemann – Short Talk**
Max Planck Institute for Molecular Cell Biology and Genetics, DE
Membrane invaginations reveal sites of force generation in C.elegans embryos

19.00 Dinner

20.00-22.00 Poster Session: Drinks Sponsored by Roche

Tuesday 26 May

Session 5

Chair: Hay-Oak Park, Ohio State University, US

09.00-09.35 **Peter Devreotes**
John Hopkins University, US
Signaling Networks for Chemotaxis

09.40-10.15 **Gaudenz Danuser; Roche Lecture**
The Scripps Research Institute, US
Dynamic interactions of actin cortex and endocytosis

10.20-10.40 Coffee break

10.45-11.20 **Jürgen Knoblich**
Institute of Molecular Biotechnology (IMBA), AT
Asymmetric cell division and proliferation control in the Drosophila and mouse nervous system

11.25-12.00 **Edwin Munro**
University of Washington, US
Intertwined mechanical and biochemical feedback loops control polarity establishment and maintenance in C. elegans

12.05-12.25	Hernan Lopez-Schier – Short Talk Centre for Genomic Regulation, ES *Afferent Neurons of the Zebrafish Lateral Line Are Strict Selectors of Hair-Cell Orientation*
12.30	Lunch
14.00	Half-day excursion to Girona
19.00	Dinner
20.00-21.00	Forward Look Plenary Discussion

Wednesday 27 May

Session 6

Chair: Maria Montoya, Centro Nacional de Investigaciones Cardiovasculares, ES

09.00-09.35	Yohanns Bellaiche ; EMBO YIP lecture Institut Curie, FR *E-Cadherin trafficking in Drosophila epithelial cells*
09.40-10.15	Keith Mostov University of California, US *Morphogenesis of multicellular epithelial structures*
10.20-10.40	Coffee break
10.45-11.20	Kozo Kaibuchi Nagoya University, JP *Axon formation and polarized vesicle transport*
11.25-12.00	Ian Macara University of Virginia, US *Par Proteins, Polarity and Morphogenesis*
12.05-12.25	Clark Wells – Short Talk University of Indiana , US *The Adaptor Protein Amot Mediates the loss of Apical Polarity by directly tethering Apical Polarity proteins to Trafficking Pathways*
12.30	Lunch
14.45-15.15	Coffee break

Session 7

Chair: Sven van Ijzendoorn, University of Groningen, NL

15.20-15.55	François Schweisguth Institut Pasteur, FR *Regulation of the trafficking and signaling activity of Notch ligands in Drosophila*
16.00-16.35	Thomas Lecuit Université de la Méditerranée, FR *Lecture*

16.40-17.15	Enrique Rodriguez-Boulan Cornell University, US *To AP1B or not to AP1B: the natural story of a Polarity Adaptor*
17.20-17.40	Rytis Prekeris – Short Talk University of Colorado Denver, US *Rip11/FIP5 and Rab11 Binding to Sorting Nexin 18 Regulates Apical Endocytic Protein Transport in Polarized Epithelial cells*
17.45-18.05	Claire Hivroz – Short Talk Institut Curie, FR *Cdc42-dependent remodeling of the cytoskeleton at the immune synapse is required for interferon-gamma secretion*
18.10-18.30	Sander van den Heuvel – Short Talk Utrecht University, NL *Control of Symmetric versus Asymmetric Cell Division in a C. elegans Epithelium*
18.35-18.55	Jordi Casanova – Short Talk Institut de Biologia Molecular de Barcelona (CSIC) / IRB, ES Cell *Adhesion and Cytoskeleton Organization in Tracheal Morphogenesis*
19.20	Before Dinner Cocktail (Dali Bar)
20.00	Conference Dinner (Salon Goya)

Thursday 28 May

Breakfast & Departure

Abstracts, Posters & Short Oral Presentations

There will be no short talks other than those listed on this Final Programme.
All accepted posters are listed on the Poster List.
Recommended poster size is 140 cm high x 100 cm wide. Use letters and drawings that can be read from approximately 100 cm distance.

Poster prizes sponsored by **EMBO, Trends in Cell Biology Journal** *and* **Nature Cell Biology Journal** *will be awarded at the end of the conference.*

Abstract

The C. elegans ARF-GEFs AGEF-1 and GBF-1 are essential for early embryogenesis

Karin Bernadette ACKEMA *and Anne Spang*
(Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland)

ADP-ribosylation factors (ARFs) play critical roles in membrane traffic within eukaryotic cells by initiating the recruitment of various coat proteins and by modulating the activity of several lipid-modifying enzymes. ARFs are cytosolic in the inactive GDP-bound form, and their activation by exchange of GDP for GTP is linked to a conformational change, which link them to membranes. ARF activation is catalyzed by the SEC7 domain of ARF guanine nucleotide exchange factors (ARF-GEFs). Two sub-families of ARF-GEFs operate at the Golgi complex: the BIG proteins (BIG1 and 2) and GBF1. BIGs and their yeast ortholog Sec7p localize to the trans Golgi, whereas GBF1 and its yeast orthologues Gea1/2p associate with the cis-Golgi. In C. elegans only one orthologue of BIG1 and BIG2 has been identified, AGEF-1, which apparently interacts with the C. elegans orthologue of class I ARFs. No detailed analysis on GBF-1 has been performed to date. Yet, various RNAi screens established both GBF-1 and AGEF-1 were required for embryogenesis.
Our lab is interested in exploring the function and mechanism of intracellular trafficking during early embryonic development and are currently investigating the role of AGEF-1 and GBF-1 in early embryogenesis. Knockdown by RNAi of either ARF-GEF resulted in a disturbed Golgi structure, eggshell-formation defects and failure in polar-body extrusion during meiosis. Moreover, while the cleavage furrow was properly formed initially in the one-cell embryo, it retracted just before completion of cell division, leading to a cytokinesis defect. The cytokinesis defect strongly resembles the phenotype of other known secretion mutants. The effects on intracellular transport by RNAi of either AGEF-1 or GBF-1 were confined to secretion defects, because endocytosis of yolk protein was unaffected in oocytes, while its secretion from the gut into the body cavity was severely compromised. Therefore, the roles of AGEF-1 and GBF-1 are conserved in eukaryotes. However, our study also revealed an unanticipated and novel role of AGEF-1 in meiosis, because, agef-1 (RNAi) showed a significant delay during meiosis in the zygote. This delay could not be correlated to the polar body extrusion phenotype and may indicate a novel role of ARFs in meiosis.

Polarized cell rearrangements orient the axis of planar polarity in the wing epithelium of Drosophila

Benoit AIGOUY*, Reza Farhadifar+, Andreas Sagner*, Jens-Christian Roeper*, Douglas B. Staple+, Frank Julicher+ and Suzanne Eaton**
*MPI-CBG +MPI-PKS
(Pfotenhauerstrasse 108, 01307 Dresden, Germany)*

To form well-functioning organs, cells must coordinate their planar polarity with the overall shape of the tissue. The planar cell polarity (PCP) pathway is a conserved mediator of this process in vertebrates and invertebrates. In the Drosophila wing epithelium, hairs are aligned with the long, proximal-distal axis of the wing and point distally. Hair orientation is specified by polarized adhesive complexes composed of PCP proteins, which change from a radial to a distal orientation prior to hair outgrowth. The mechanisms that orient PCP domain polarity are not understood. By quantitatively analysing long-term time-lapse movies of this process, we show that dramatic cell rearrangements reshape the wing as PCP domains polarize. Contraction of the wing hinge stretches wing blade cells along the proximal-distal axis, inducing specific patterns of cell flow, shear and cell division that elongate and narrow the wing blade. In addition to reshaping the wing, these flows produce both hexagonal packing of wing epithelial cells and the alignment of PCP proteins along the proximal-distal axis. We combine this quantitative analysis with a theoretical multi-scale approach. Simulations of cell rearrangements in a vertex model show that shear can reorient PCP polarity. We show that the observed large-scale pattern of shear, in combination with the initial orientation of PCP domains, can realign PCP domains with the proximal-distal axis. Consistent with this result, experiments show that planar polarity is reoriented by altering flow patterns either genetically or by wounding. Thus, a single cue both orients cell rearrangements and ensures that intracellular planar polarity is coordinated with the final shape of the wing.

Beta 1 integrins control lumen formation and glandular epithelial morphogenesis through ILK

Nasreen AKHTAR, *Emma T Lowe, Julia Cheung and Charles H Streuli*
Faculty of Life Sciences, University of Manchester, Oxford Rd, Manchester M13 9PT, UK.
(Michael-Smith building, Oxford Rd, M13 9PT Manchester, United Kingdom)

Integrin-mediated adhesion to the extracellular matrix plays a critical role for the development and function of mammary epithelia. This tissue is organised into a branching network of tubes with alveoli (acini) composed of a monolayer of luminal epithelia surrounded by myoepithelia and a basement membrane (BM). The luminal epithelia within acini are polarised such that the apical secretory face is adjacent to a central cavity and the basal surface is in contact with the BM. During pregnancy the luminal cells proliferate in a highly organised manner such that the overall architecture and polarity of acini is maintained. A possible mechanism that would allow such coordinated growth is correct spindle alignment during cell division such that cells always divide in the plane of the matrix. We demonstrate that beta 1 integrins play a critical role in this process. Conditional deletion of beta 1 integrins with CreLoxP in vivo and in primary cultures severely impaired acinar morphogenesis. Beta 1 integrin null acini displayed either a single collapsed lumen or multiple small lumens with cells protruding into the cavity and in more severe cases a complete absence of a lumen. Analysis of apical markers in primary cultures revealed mislocalisation of the aPKC-Par3-Par6 complex to the basal domain indicating that beta 1 integrins are essential for correct orientation of apical polarity.

Downstream of integrins we have identified integrin-linked kinase (ILK) as a key mediator in the development of mammary acini with a single cell-free lumen. ILK was displaced from the cell membrane in integrin null acini, moreover, conditional deletion of ILK both in vivo and in cultured acini resulted in abnormal acinar morphogenesis with multilayering. We are currently investigating the possibility that integrins and ILK co-ordinately control apical polarity and spindle orientation during cell division and thereby allow polarised growth of mammary acini. Interestingly, a similar dismorphology is frequently observed in early breast cancers such as ductal carcinoma in situ (DCIS) raising the possibility that integrins and ILK might be disengaged with apical markers causing spindle misalignment and cell growth into the lumen.

Recognition, internalization and degradation of apoptotic cells in C. elegans

Johann ALMENDINGER, *Kimon Doukoumetzidis, Jason M. Kinchen, Kodi S. Ravichandran and Michael O. Hengartner*

(Institute of Molecular Biology, University of Zurich, CH-8057, Zurich, Switzerland. Beirne Carter Center for Immunology Research, University of Virginia, Charlottesville, VA 22908, USA)

Apoptotic cell clearance is an important process during development, tissue homeostasis and wound healing of multicellular animals. Removal of apoptotic cells or engulfment can be broken down in several steps such as corpse recognition, internalization, phagosome maturation and degradation within the phagolysosome. Defects in the removal and degradation of apoptotic cells can lead to autoimmune diseases due to the exposure of autoantigens. Studies over the last decades in C. elegans let to the identification of seven major genes regulating corpse internalization. These genes act in two partially redundant parallel pathways comprising ced-1, ced-6, ced-7 and ced-2, ced-5, ced-12 respectively to activate ced-10. Additionally, four major genes dyn-1, vps-34, rab-5 and rab-7 orchestrate maturation of phagosomes containing apoptotic cells towards cell corpse degradation within phago-lysosomes. Here we present new findings gained by stuying a candidate gene identified in a reverse genetic screen, that most likely plays an important role in regulating membrane dynamics during the degradation of apoptotic cells.

Poster 5

Regulation of lumen formation and transcytotic transport by a novel member of the formin family

Ricardo Madrid[1], Juan F. Aranda[1], Leandro Ventimiglia[1], Laura Andrés[1], Sergio Gómez[1], Alberto Jiménez[1], Jennifer A, Byrne[2], **Miguel A. ALONSO[1]**

[1]Centro de Biología Molecular Severo Ochoa. CSIC/UAM. Cantoblanco. 28049-Madrid, Spain. [2]Oncology Research Unit and the University of Sidney Department of Pediatrics and Childs Health, The Children s Hospital, Westmead, NSW, 2145, Australia
(Centro de Biología Molecular Severo Ochoa, Nicolas Cabrera, 1, 28049 Madrid, Spain)

In epithelial cells, proteins destined for the apical surface travel directly from the Golgi or indirectly by making a detour to the basolateral surface before being internalized and transported to the apical membrane in specialized endosome carriers in a process known as transcytosis. Hepatocytes constitute a simple model for apical transport studies as the indirect route constitutes the sole mechanism for apical targeting of proteins attached to the membrane by a single anchor. MAL2, an integral membrane protein of the MAL family, is an essential element of the machinery for basolateral-to-apical transcytosis in hepatoma HepG2 cells (de Marco et al., 2002). At steady state, MAL2 predominantly distributes at the apical surface but inhibition of transcytosis in MAL2-silenced cells does not produce cargo arrest early in the transcytotic pathway. Analysis of MAL dynamics revealed that a fraction of apical MAL2 redistributes into peripheral endosome elements to concentrate cargo internalized from the baolateral membrane. Then, these MAL2+ endosomes progressively fuse and move towards the apical surface for cargo delivery (de Marco et al., 2006). MAL2 dynamics, therefore, explains the apparent paradox whereby MAL2 silencing results in the accumulation of cargo in endosome elements distant from the apical surface as if MAL2 were mediating transcytosis at a distance.
Diaphanous-related formins (Drfs) bridge G-protein signals and the actin and tubulin cytoskeletons via their ability to bind active small Rho GTPases. Unlike actin-related protein 2/3 (Arp2/3) complex that generates branched actin filaments, Drfs are involved in formation of linear actin filaments. Drfs have been found to modulate a number of intracellular processes such as endosome motility, microtubule stabilization and cytokinesis.
To obtain insights into the mechanism of transcytosis, we have carried out a search for proteins that interact with MAL2 and identified Drf5, a novel formin with a Drf domain organization, as a MAL2 partner. MAL2 and a fraction of Drf5 colocalized at the apical surface of HepG2 cells. Silencing of Drf5 decreased MAL2 dynamics, slowed down transcytotic transport and diminished the number of intercellular lumens. Drf5, therefore, controls lumen formation and apical transcytosis by regulating MAL2 trafficking in HepG2 cells.

- de Marco, M.C., Martin-Belmonte, F., Kremer, L., Albar, J.P., Correas, I., Vaerman, J.P., Marazuela, M., Byrne, J.A., and Alonso, M.A. (2002). J. Cell Biol. 159, 37-44.
- de Marco, M.C., Puertollano, R., Martínez-Menárguez, J.A., and Alonso, M.A. (2006). Traffic 7, 61-73.

Angina, the Drosophila homolog of the ARF-GEF GBF1, is required for epithelial tube size control and function of the Golgi Apparatus

Kristina ARMBRUSTER *and Stefan Luschnig*
(Institute of Zoology University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland)

The function of tubular organs, like the lung or the kidney, depends on the proper dimensions of epithelial tubes. We are using the tracheal system of Drosophila as a model to study cellular mechanisms controlling epithelial tube size. Tracheal cells secrete an extracellular matrix (ECM) into the apical lumen as it expands during embryogenesis. ECM secretion is essential for tracheal tube expansion. We have performed a screen for mutations affecting tracheal tube size. Here we show that mutations in the Drosophila homolog of the ARF-GEF GBF1, angina (aga), affect tracheal tube size and protein secretion and lead to a breakdown of the Golgi Apparatus. Like human GBF1, GFP-Aga localizes to the cis Golgi. We show that the C-terminus of Aga is required for Golgi localization. Furthermore, human GBF1 is able to rescue the aga tracheal phenotype. Interestingly, additional copies of aga lead to a phenocopy of the secretion and lumen dilation defect, while Golgi morphology remains unaffected. Our results suggest that the ARF-GEF Aga controls epithelial secretion and tracheal tube size by regulating the rate of retrograde traffic from the Golgi to the ER. Moreover, our data indicate an important role of Aga/GBF1 in the maintenance of Golgi structure.

mRNA trafficking in neurons in context of cell polarity

Varuzhan BALASANYAN
(1050 Child's Way, RRI 208, 90089 Los Angeles, United States)

Neurons are highly polarized cells with well-defined, intrinsically unique, and functionally distinct dendritic and axonal extensions. Many mechanisms are evolved to maintain that unique polarity (created earlier during development of the neuronal cells), and one of those mechanisms is mRNA trafficking between cell body and dendrites/axons. Significant evidence has accumulated recently to support the idea of remote protein synthesis in distal compartments of dendrites and axons. How mRNA targeted differentially to dendrites or axons is still unclear, but there is evidence of implication of 3'- and 5'-untranslated regions ("zipcodes") of mRNA in this process.
Here we are providing tool for tagging mRNA in living context of the cells, and future implication of this tool to elucidate differential trafficking of different mRNA in neurons.

Poster 8

A CD317(tetherin)/RICH2 complex plays a critical role in the organisation of the sub-apical actin cytoskeleton in polarised epithelial cells

George BANTING
(University Walk, Bristol, United Kingdom)

Ruth Rollason, Viktor Korolchuk, Clare Hamilton, Mark Jepson and George Banting
Dept. of Biochemistry, University of Bristol, Bristol BS8 1TD, UK

CD317/tetherin is a lipid raft associated integral membrane protein with a novel topology. It has a short N-terminal cytosolic domain, a conventional transmembrane domain, and a C-terminal GPI anchor. We now show that CD317 is expressed at the apical surface of polarized epithelial cells where it interacts, indirectly, with the underlying actin cytoskeleton. CD317 is linked to the apical actin network via the proteins RICH2, EBP50 and ezrin. Knocking down expression of either CD317 or RICH2 gives rise to the same phenotype: a loss of the apical actin network with concomitant loss of apical microvilli, an increase in actin bundles at the basal surface, and a reduction in cell height without any loss of tight junctions, transepithelial resistance or the polarized targeting of apical and basolateral membrane proteins. CD317 thus provides a physical link between lipid rafts and the apical actin network in polarized epithelial cells and is crucial for the maintenance of microvilli in such cells.

Poster 9

Characterization of the MAL2 positive compartment in oligodendrocytes

Raquel BELLO-MORALES *and José Antonio López-Guerrero*
(Centre of Molecular Biology Severo Ochoa, C/ Darwin, 2, Cantoblanco, 28049 Madrid, Spain)

Oligodendrocytes, the myelin-producing cells of the central nervous system, segregate different surface subdomains at the plasma membrane as do other differentiated cells, such as polarized epithelia and neurons. To generate the complex membrane system that characterizes myelinating oligodendrocytes, large amounts of membrane proteins and lipids need to be synthesized and correctly targeted. In polarized epithelia, a considerable fraction of apical proteins are transported by an indirect pathway involving a detour to the basolateral membrane before being internalized and transported across the cell to the apical membrane by a process known as transcytosis. The Apical Recycling Endosome (ARE) or the Subapical Compartment (SAC) of hepatocytes, are intracellular trafficking stations involved in the transcytotic pathway. MAL2, an essential component of the machinery for basolateral-to-apical transcytosis, is an ARE/SAC resident protein. Although MAL2 expression has been detected in the KG-1C oligodendroglial cell line (Bello-Morales et al., 2005), no studies on MAL2 distribution has been reported in these cells yet. We are investigating the expression of MAL2 in oligodendrocytic cell lines, as well as its regulation during cell differentiation. In addition, the characteristics of the MAL2 positive compartment of differentiated cells and its similarity with the ARE/SAC of epithelial cell lines have been studied. We suggest that the MAL2 positive compartment in oligodendrocytic cells could be a structure analogous to the ARE/SAC and might have an important role in the sorting of proteins and lipids for myelin assembly during oligodendrocytes differentiation. Ref. Bello-Morales R., Fedetz M., Alcina A., Tabarés E. and López-Guerrero J. A. (2005). High susceptibility of a human oligodendroglial cell line to herpes simplex type 1 infection. J. Neurovirol. 11:190-8.

Poster 10

AP-1 clathrin adaptor complex controls the localization of the Notch-signalling regulator Sanpodo in Drosophila sensory-organ precursor cells

Najate BENHRA
(Institut de Génétique et Développement de Rennes - UMR6061 CNRS, 2 av du Pr Leon Bernard - CS 34317, 35043 Rennes, France)

During metazoan development cell fate diversity is in part generated via asymmetric cell division, in which mother cells divide to produce two daughter cells with distinct developmental potential. In Drosophila, the sensory organ precursor cell called pI divides four times asymmetrically to generate the sensory organ lineage. During pI division, the cell fate determinant Numb segregates asymmetrically at the anterior cortex and is unequally inherited by the anterior daughter cell. Numb is proposed to negatively regulate Notch signalling pathway by promoting the endocytosis of the four-pass transmembrane protein Sanpodo (Spdo). Thus the anterior pI daughter cell adopts the pIIb fate. By contrast, in the posterior cell Spdo localizes at the plasma membrane where it physically interacts with the Notch receptor to specify the Notch-dependent fate. This cell adopts the pIIa fate. We recently isolated the Drosophila AP47 gene encoding the μ1 subunit of the AP-1 clathrin adaptor complex as a novel regulator of Notch signalling in these epithelial cells. We report that loss of function of AP47 causes an early embryonic lethality indicating that AP47 is an essential gene as previously reported in C. elegans and mice. In mosaic flies, loss of function of AP47 causes a pIIb to pIIa cell fate transformation. Similar results were also obtained using tissue specific RNAi against any of the four subunits of the AP-1 complex. The unequal segregation of cell fate determinants is unaffected in cells mutant for AP-1. The steady state distribution of Delta and Notch remain unaffected in AP47 mutant cells. However, we observe that Spdo trafficking is strongly altered. In cells mutant for AP47, Spdo accumulates at the apical surface of the pI cell and that of both pI daughter cells, where it colocalizes with Notch. We propose that AP-1 regulates cell fate by inhibiting the plasma membrane localization of Spdo and thereby preventing the activation of Notch signalling in the anterior pI daughter cell.

Systems view on morphogenesis: Multiple phase transitions control lumen formation

Ulrich BERGE*1*, Aldo Ferrari[1]*, Alexey Veligodskiy[1]*, Miriam S. Lucas[2], Ruth Kroschewski[1]*

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**contributed equally to this work*

Organs occur reproducibly with similar shapes and are elaborate cell communities that perform specialized functions. Each individual organ-constituting cell thus plays an important role in shaping organs. Therefore, organogenesis depends on the ability of each cell to control its own shape and contacts with its neighbours. In mammalian organs, such as kidney and lung, epithelial cells assemble into specialized interfaces between the organism and the outside world. To achieve this, they establish an apical surface, facing the lumen, a contact surface, establishing the communication with neighbouring cells, and a basal surface, touching the surrounding extra-cellular matrix. These polarized cells form mainly two organ building blocks, cysts and tubules, each characterized by a central volume, a lumen. How is this space generated in the first place? Understanding lumen formation reflects understanding of how cells polarize during organogenesis and how regulation of cellular mechanics coordinates cell shape changes. The dog kidney epithelial cells, MDCK, reproduce cyst formation in vitro. We thus aim to understand how physical concepts influence biochemical processes in the MDCK model system. Using quantitative live-cell imaging, we investigated the development of single MDCK cells into aggregates with lumen. In two-cell aggregates, membrane insertion into the contact surface established a preapical patch (PAP), characterized by the presence of the apical marker gp135, microvilli and the absence of E-cadherin. This PAP originated from a compartment with hallmarks of an apical recycling endosome, and matured through Brefeldin-A-sensitive membrane trafficking and the establishment of tight junctions around it. As a result of the activity of water and ion channels, an optically resolvable lumen was subsequently formed. Initially, this lumen enlarged without changes in aggregate volume or cell number but with decreasing cell volumes. Additionally, the ROCK1/2-myosin-II pathway counteracted PAP and lumen formation. Hence, lumen formation results from PAP establishment, PAP maturation, lumen initiation and lumen enlargement. As these phases correlate with distinct cell surface and volume patterns, our results suggest that such morphometric parameters are regulated by trafficking, ROCK-mediated contractility and hydrostatic pressure or vice versa.

Poster 12

Quantitative analysis and mathematical modeling of polarity establishment in C. elegans embryos

Simon BLANCHOUD, *Yemima Budirahardja, Felix Naef, Pierre Gönczy*
(Swiss Institute of Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Station 19, 1015 LausanneLausanne, Switzerland)

The PAR proteins, central players of cell polarity, were discovered in the nematode C. elegans and later shown to be functionally conserved across metazoan evolution. In order to better understand how interactions between these proteins result in cell polarity, we use a systems biology approach rooted in the one-cell stage C. elegans embryos as a model system.
As a first step, we focus our analysis on two PAR proteins distributed in complementary domains, PAR-6 at the anterior cortex and PAR-2 at the posterior cortex. In order to capture the spatio-temporal dynamics of these two components, we imaged a strain simultaneously expressing GFP-PAR-2 and mCherry-PAR-6 using confocal time-lapse microscopy. Preliminary analysis of the data indicates that this should enable us to obtain quantitative kinetic data on the process of polarity establishment.

To automatically analyze these time-lapse recordings, we are developing a reference coordinate system (RECOS). This program can automatically detect the eggshell and the cortex of the embryos. Using the detected cortex, the program creates the intensity profile based on the two individual fluorescent channels. This intensity is then mapped onto a reference embryo to enable consistent analysis of the data. Various image analysis methods are currently explored to improve the detection accuracy of RECOS.
Ultimately, we aim at developing and testing a robust continuous space reaction-diffusion model describing polarity establishment spatially and temporally in early C. elegans embryos. This model will be calibrated using the quantitative data extracted from the time-lapse recordings using RECOS. We expect this model to uncover general polarization principles, to be able to correctly predict know mutant behaviors and to provide a framework for further analysis.

The intraflagellar transport components KIF3A and IFT88/Polaris are required for directed migration of non-ciliated cells

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Dysfunction of primary cilia, non-motile filiform structures projecting from the surface of most cells, has lately been implicated in a broad spectrum of diseases such as retinal degeneration, polycystic kidney disease and obesity. An evolutionarily conserved intraflagellar transport (IFT) system helps sustain ciliary structure and function. Anterograde ciliary transport of multi-protein IFT complexes is maintained by kinesin II and mutation of the kinesin II subunit KIF3A or IFT proteins in mice leads to the loss of tubular geometry and cyst formation. In rat hippocampal neurons KIF3A interacts with Par-3 to mediate the outgrowth of axons, suggesting that KIF3A may have extra-ciliary functions in epithelial polarity. Abrogating ciliogenesis by inducible knock-down of KIF3A in MDCK cells resulted in normal localization of apical-basal polarity markers such as zo-1, E-cadherin and beta-catenin. Calcium-switch experiments however revealed delayed tight junction assembly after depletion of KIF3A. When grown in a collagen I matrix, KIF3A depleted cells lost the ability to polarize into three-dimensional structures and to form extensions after HGF treatment. Scratch induced wound healing of unciliated cells in 2D culture resulted in gross impairment of migration speed after KIF3A depletion, but normal scratch- and growth factor-induced activation of ERK1/2 and Rac1. Immunofluorescence revealed mislocalization of the golgi apparatus and Scrib in migrating cells. Surprisingly, also knockdown of ciliary IFT protein IFT88 (polaris) phenocopied the migration and polarity defects. Our findings uncover a novel role of the ciliary transport proteins KIF3A and IFT88 in cell migration and polarity of epithelial cells independent of the primary cilium.

Drosophila Cip4/Toca-1 integrates membrane trafficking and actin dynamics through WASP and WAVE/SCAR

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Developmental processes are intimately tied to signaling events that integrate the dynamic reorganization of the actin cytoskeleton coupled to membrane dynamics. The F-BAR-domain-containing proteins (F-BAR proteins) are prime candidates to couple actin dynamics and membrane trafficking in different morphogenetic processes. The most striking feature of F-BAR proteins is the ability to induce membrane tubules and to regulate Arp2/3 mediated actin nucleation. Here, we present the functional analysis of the Drosophila F-BAR protein Cip4/Toca1 (Cdc42 interacting protein 4/ Transducer of Cdc42-dependent actin assembly 1). Drosophila Cip4 is able to tubulate liposomes as well as membranes in Drosophila S2R+ cells. Importantly, Cip4 is able to form a complex with WASP and WAVE/SCAR and recruits both nucleating promoting factors to membrane tubules. Interestingly, Cip4-EGFP marked endosome movement driven by actin comet tails depends not only on WASP function but in particular on WAVE/SCAR function in S2R+ cells. Loss of cip4 function caused a multiple wing hair phenotype. Suppression of WAVE/SCAR function but not WASP function results in a similar phenotype. Gene dosage experiments clearly show a functional interaction between cip4 and wave/scar indicating that in vivo Cip4 can also act through WAVE/SCAR. Biochemical and functional data further support a model in which Cdc42 acts upstream of Cip4 and recruits WAVE/SCAR via Abi to control Dynamin-dependent cell polarization in the wing.

CHARACTERIZATION OF SNARE PROTEINS IN HUMAN NK CELLS

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NK cells participate in host protection by eliminating cells with altered expression of Major Histocompatibility Complex class I (MHC-I) molecules, which can result from viral infection or transformation. Even though their main cytotoxic mechanism is granule secretion, little is known about the components of the membrane fusion machinery that catalyze and regulate this kind of exocytosis. The present study explores, in human NK cells, the possible presence of one of the universal mediators of membrane fusion: the SNARE (soluble N-ethylmaleimide (NEM) sensitive factor attachment protein receptor) protein system, and examines its functional role in NK cell granule exocytosis. Up to now, we have identified numerous members of this protein family (as transcripts and as proteins) in the established cellular lines of NK cells (NK-92 and NK-L) as well as in peripheral blood human NK cells.
SNARE transcripts identified in human NK cells correspond with proteins previously identified as elements of the exocytic route in other cellular types of hematopoietic origin. Members of the three SNARE subfamilies have been identified: Syntaxin (2, 3, 4, 6, and 11), VAMP (2, 3, 7 y 8) and SNAP-25 subfamilies (SNAP-23). In addition, the expression of SNARE proteins was confirmed and their subcellular localizations were determined by confocal fluorescence microscopy. It widely emphasizes the coincident location of VAMP- 7 with cytotoxic granules, in accordance with previous studies of other authors, and as other SNAREs members are vesicles associated. In conclusion, human NK cells express several protein isoforms corresponding to the three SNARE families. This serves as starting point for identifying what SNARE proteins are forming the complexes that mediate the secretion of cytotoxic granules and to see if they are different from the SNARE complexes needed for cytokine secretion.
In order to evaluate the role of the identified SNARE proteins in granule exocytosis our current work focuses on optimizing inhibition assays.

Poster 16

Role of the apical Par-aPKC complex in the imaginal discs

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The aim of the present work is to study the regulation and function of the polarity determinant DaPKC in the epithelial cells of the imaginal discs of Drosophila. Our analysis of the subcellular distribution of the different polarity determinants in the wing disc epithelium has revealed subtle differences to their localization in the embryonic epithelium. Thus, whereas apical DaPKC, Crumbs (Crb) and Echinoid (Ed) and basolateral Discs large (Dlg) and Scrible (Scrib) maintain their non-overlapping subcellular distributions, PATJ and Crb overlap with DaPKC but also extend further apically and basally, respectively. In the discs, proper activity of polarity proteins, tissue organization and growth are closely related. Thus, loss of function of basolateral polarity markers such as Dlg), Lethal giant larvae (Lgl) or Scrib causes strong tissue overgrowth. We have found that clones of DaPKC null cells are scarcely viable and are extruded from the epithelium, accumulating high levels of actin and showing delocalization of Armadillo, D-E cadherin and Ed, indicative of compromised adhesion. In addition, Crb is lost from the membrane although distribution of Dlg and Scrb is not modified. Over-expression of membrane targeted DaPKC (UAS-DaPKCCAAXWT) in the wing disc causes depletion of Ed or Arm and tissue overgrowth. This overgrowth is similar to that found in scrb/lgl/dlg mutant discs, reinforcing the proposal of functional antagonism, described in the embryo, between apical and basolateral determinants. However, DaPKCCAAXWT expressing clones cause overgrowth whereas clones of cells mutant for basolateral identity determining genes fail to grow and are eliminated from the disc epithelium. Constitutive activation of DaPKC is also associated with trafficking defects in the wing disc epithelium. Thus, DaPKCCAAXWT over-expressing cells show enlarged accumulation of the early endosomal marker Hrs and abnormal dextrane uptake. We are analyzing to what extent defective trafficking affects the signalling pathways that operate in the wing disc and their contribution to the mutant phenotype. We propose DaPKC to behave as a protooncogene in Drosophila, as it has been shown in vertebrates, which should play a key role in the regulation of cell proliferation and in the maintenance of epithelial integrity in the developing wing disc.

Poster 17

Cell adhesion and cytoskeleton organization in tracheal morphogenesis

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In the lab, we are investigating the mechanisms and cellular events that underlie tracheal cell morphogenesis and how these mechanisms are regulated. Although much is known about the genes required for the determination of the tracheal cells, their morphogenesis is poorly characterized at the cellular level. On the one hand, we are addressing the behaviour of tracheal cells in migration. Epithelial cells are tightly coupled via distinct intercellular junctions. However, during morphogenesis some epithelial cells exchange places and move in a spatially oriented manner. In particular, not much is known about how the different genes and signalling pathways have an effect on the cytoskeleton components that induce their migration. Our results suggest that intercalation and migratory behaviour of tracheal cells depend on a compromise between the migratory signal they receive and modulation of their adhesion On the other hand, we are currently realizing a detailed description of the cellular shape modification and cytoskeleton reorganization that accompany tracheal cell morphogenesis. Based on this description, we explore the contribution of the signalling pathways and transcription factors involved by precisely analyzing the phenotypes induced at the cellular level by mutants for these factors.

Hepatocyte polarity and extracellular matrix-cell contacts

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Most liver functions are insured by hepatocytes. These polygonal epithelial cells present a complex polarity, each hepatocyte having several basolateral and apical (biliary) poles. This polarity is very difficult to maintain in vitro and polarized lines are very rare. The aim of this work was to evaluate, using the polarized WIF-B9 line (that forms simple bile canaliculi) and its unpolarized parent Fao, the role of extracellular matrix (ECM)-cell interactions on the establishment and maintenance of the typical hepatocyte polarity.

Cells were cultured in different configurations : either on a thin layer of various ECM components (collagen I, collagen IV, laminin, fibronectin, ..) or on, in sandwich, and inside a gel of collagen I, Matrigel, or Puramatrix. Structural cell polarity was evaluated by immunolocalization of twenty apical (canalicular), basolateral, tight junctional and cytoskeletal proteins, and by confocal laser microscopy and electron microscopy. Functional polarity was tested by evaluating the transport of organic anions and biliary salts. Trafficking of apical proteins was studied by following the itineraries of antibody-labeled proteins.

No polarisation of Fao cells was induced whatever the conditions used. In contrast significant changes in WIF-B9 polarity were induced. The culture inside Matrigel (but not in sandwich or on Matrigel), and to a lesser extent in gel of collagen I, led to a more rapid polarisation and an improvement of WIF-B9 polarity with the formation of functional long and branched canalicular structures, reminiscent of those formed in vivo. In contrast the culture on collagen IV (and to a lesser extent on Puramatrix) induced a regression of WIF-B9 polarisation with appearance of hudge intracellular vacuoles rich in microvilli, in which were localized apical proteins (DPPIV, Mdr, Mrp2,..) and efficiently transported bile salts and organic anions. Neither tight junctional proteins nor basolateral markers were localized in these vacuoles. Dynamic studies showed that apical proteins in WIF-B9 cells cultured on collagen IV were first delivered to basolateral domain, as in normal hepatocytes, before being routed to vacuoles, where they stayed and did not recycle. These results strongly suggest that collagen IV-induced vacuoles correspond to internal functional bile canalicular structures.

In conclusion, ECM-cell interactions are able to modulate typical hepatocyte polarity expressed by WIF-B9 cells and can lead to the formation of intracellular apical structures. This polarity can be improved with Matrigel, a biological already well formed matrix, provided cells were embedded in this matrix.

Poster 19

Control of apical cell shape remodelling: involvement of ZP proteins during epidermal cell morphogenesis

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How developmental programs act to ultimately modify the form of individual cells remains poorly understood, especially little is known on the mechanisms remodelling the apical compartment of epithelial cell. The drosophila embryonic epidermis is composed by a monolayer of epidermal cells adopting two distinct fates: epidermal cells producing thick hair-like structures called denticles and smooth cells. This basic cell fate pattern of alternating denticles and smooth cells depends of the activity of the Ovo/Shavenbaby (Svb) transcription factor which controls epidermal apical cell shape remodeling to promote denticle formation. Therefore, the identification of svb targets provides a breakthrough toward the cellular effectors involved in this process. In addition to cytoskeletal regulators, we identified new svb targets, encoding a family of extracellular proteins. These proteins belong to the conserved Zona Pellucida (ZP) protein family, encoding membrane anchored secreted proteins. By generating mutant for each ZP gene, we showed that denticle formation relies on the activity of several ZP proteins, each of them providing a localised function for denticle formation. Ultra structural analyses reveal that loss of a given ZP protein disrupts ECM-cell membrane interactions in a limited apical region. In the forming denticle, ZP proteins accumulation is highly ordered in different discrete apical sub domains along an apical-basal axis where their activities are required, thus defining an unusual heterogeneously ordered apical extracellular microenvironment. A ZP mutant also leads to defects of Crumbs and Ecad accumulation, suggesting a cross talk occurring between ZP proteins and cell polarity components. We propose the existence of a heterogeneous/sequential assemblage of various distinct ZP proteins, linking extracellular proteins to the cell junction polarity complexes.

Neisseria meningitidis, the causative agent of cerebrospinal meningitis, recruit the Par3/Par6/PKCzeta polarity complex to open the junctions between brain endothelial cells

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Type IV pili (Tfp) mediate the initial interaction of many bacterial pathogens with their host cells. In the case of Neisseria meningitidis (Nm), the causative agent of cerebrospinal meningitis, Tfp mediated adhesion to brain endothelial cells is a prerequisite to the crossing of the blood brain barrier (BBB). Little is known about the mechanism following this initial event allowing the bacteria to cross the BBB and to invade the meninges. We demonstrate that Tfp-mediated adhesion of Nm recruits the polarity complex Par3/Par6/PKCzeta which plays a pivotal role in the establishment of eukaryotic cell polarity and the formation of intercellular junctions. The recruitment of the polarity complex is followed by the re-routing of junctional proteins at the site of bacterial cell interaction and the formation of ectopic intercellular junctional domains. This leads to an increase of the permeability of human brain endothelial cell monolayers. These data reveals a mechanism by which a human pathogen can open the BBB.

The transcription factor ZONAB is a master switch between proliferation and apical differentiation in kidney proximal tubular cells (PTC)

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A central feature of embryogenesis and tissue repair is the switch between proliferation and differentiation. The transcription factor, ZONAB, reported to translocate into nuclei to promote cyclin D1/PCNA expression and cell proliferation, or to be sequestered by ZO-1 at tight junctions, qualifies as a candidate polarity regulator. We have investigated the expression and effects of ZONAB in three complementary models: kidney cancer, kidney development and in vitro polarization of OK cells, a well-established proximal tubular cell (PTC) line. In silico analysis of clear cell renal carcinoma microarray databases and quantitative RT-PCR on additional samples systematically disclosed increased ZONAB expression and proliferation markers, inversely correlating with expression of differentiation markers (the tandem apical endocytic receptors, megalin and cubilin, and villin). During ontogeny, E13.5 mice embryos showed a strong ZONAB labelling by in situ hybridization in kidney cortical primary tubules. Expression profiles for ZONAB mRNA and protein during further kidney development revealed that disappearance of ZONAB and PCNA was concomitant with appearance and increased expression of megalin and cubilin. The same opposite expression profile was observed upon in vitro polarization of OK cells and was functionally reflected by enhanced apical receptor-mediated endocytosis of megalin and cubilin ligands. To address if the opposite regulation between ZONAB and endocytic receptors was a cell-autonomous response that could be distinguished between adjacent cells, OK cells were sparsely plated to form small colonies, where junctional belts are continuous only around central cells but are interrupted at the outer margin of peripheral cells. The latter showed strong ZONAB nuclear staining and entry into S-phase (EdU incorporation) but minimal megalin expression, while neighbouring central cells were devoid of nuclear ZONAB, did not enter S phase and abundantly expressed megalin. Transient transfection with ZONAB repressed megalin and cubilin promotors in luciferase reporter assays, which was reversed by ZO-1 co-transfection, demonstrating the specificity of the interaction. Binding of ZONAB to endogenous megalin and cubilin promoters was confirmed by chromatin immunoprecipitation. Stable ZONAB transfection strongly repressed megalin and cubilin expression and endocytic function. ZONAB overexpression also repressed villin expression and alpha-tubulin acetylation, as reflected by impaired maturation of the brush border and the primary cilium. Interestingly, polarity down-regulated ZONAB transcription and induced its proteasomal degradation. We propose that ZONAB is a master transcription factor, acting as a switch between proliferation and differentiation via direct and simultaneous opposite effects, and down-regulated by polarity at transcriptional and post-translational levels.

Role of retromer in traffic of the β-site amyloid precursor protein (APP) cleaving enzyme (BACE) and β-APP

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Retromer is an evolutionary conserved protein complex that mediates endosome-to-Golgi retrieval of receptors for lysosomal hydrolases. It is constituted by five Vacuolar protein sorting (Vps) subunits organized into two sub-complexes: one formed by Vps26-Vps35-Vps29, in charge of cargo recognition, and another one comprising the two sorting nexins SNX1 and SNX2, which deforms the membrane to ensure cargo sorting. Research in different organisms indicates that retromer participates in the sorting of additional proteins. Two of such putative cargos are the amyloid precursor protein (β-APP) and the β-site APP cleaving enzyme (BACE or β-secretase), which hydrolyzes β-APP leading to production of the neurotoxic amyloid β peptide and progression of Alzheimer disease. Here, we study the connection between retromer and BACE-APP traffic in two models of polarized cells, neurons and Madin-Darby canine kidney (MDCK) cells, which share some mechanisms for polarized sorting.
Immunohistochemical detection of retromer subunits in adult mouse brain revealed a similar pattern of regional distribution, including neurons of the cerebral cortex, hippocampus (pyramidal cells), basal ganglia, substantia nigra, striatum and cerebellum. In cerebellum, an inside-out gradient of retromer expression was observed, with apparent staining in dendrites of Purkinje cells but not in their axons. In primary cultures of neurons differentiated in vitro from subventricular zone neurospheres, ectopically expressed BACE partially co-localized with Vps26 in the soma as well as in vesicles traveling along neurites.
The use of MDCK cells as a model system has allowed the generation of cell lines stably co-transfected with BACE and APP. Co-immunoprecipitation of Vps35 with myc-BACE and APP suggests an interaction of retromer with these proteins in vivo. Treatment of MDCK cells with the phosphoinositide 3-kinase (PI3K) inhibitor LY294002, which affects retromer’s function, alters the association between myc-BACE and APP. Inhibiting PI3K, localization of BACE in endosomes is increased, suggesting its retention in endosomes in the absence of retromer’s function. These results support a role of retromer in traffic of BACE and/or APP.

Decoding heparan sulfate: what works on what, and where?

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While the best recognized role of endocytosis in signaling is to down-regulate the signal by sending the receptor and /or the ligand to degradative compartments such as lysosomes, it has now become clear that endocytosis also contributes to activation of signaling, modulating interactions between signaling molecules and their inhibitors, regulating receptor presentation at the cell surface, contributing to the formation of morphogen gradients and even providing localized endosomal environments where signaling takes place. Thus, the pathways of vesicular trafficking are emerging as extremely important for the spatial and temporal fine-tuning of developmental signaling, and there is circumstantial evidence that the cell surface heparan sulfate proteoglycans have specific roles in this respect. Research addressing the functions of the syndecans in signaling has mostly centered on their presumptive roles as cell surface heparan sulfate-acceptors/receptors of extracellular matrix proteins and soluble growth factors. It appears now that, side by side with integrins and tyrosine kinase receptors, the core proteins of the syndecans contribute significantly in signaling and are regulated by signaling. We have evidence that, via the PDZ-protein syntenin, the syndecans are connected with PIP2, a phophoinositide that in concert with Arf6 regulates the organization of the actin cytoskeleton and vesicular transport. Cell surface recycling of syndecan and syndecan cargo, such as adhesion molecules, FGF and FGFR depend on syntenin and Arf6, potentially identifying a novel signaling function for these ‘co-receptors’. Indeed, it is generally accepted that FGF-FGFR signaling depends on specific ‘templates’ that are ‘encoded’ in the structure of the heparan sulfate that is present at cell surfaces. Surprisingly, cell surface heparan sulfate appears to be quite ineffective, even inhibitory in terms of FGF signaling. Yet, mammalian heparanase, an endosomal/lysosomal endo-glucuronidase that cleaves heparan sulfate at a limited number of sites, converts such inhibitory heparan sulfate into a template that stimulates FGF signaling. Mammalian heparanase is upregulated in inflammatory cells and in cancer cells. Using surface heparan sulphate proteoglycan, low density lipoprotein receptor-related proteins and mannose-6-phosphate receptors, most cells can also capture secreted pro-heparanase and direct the inactive precursor protein to late endosomes / lysosomes where it is activated by proteolytic processing. Strikingly, when syntenin is disconnected from PIP2, mammalian heparanase accumulates along with syndecan and FGFR in recycling endosomes. We therefore suspect that heparan sulfate ‘instructions’ may also be encrypted into irrelevant contexts, and that the ‘heparanome’ may need to be ‘deciphered’ by endocytic decoders.

Galectin-3, a novel centrosome-associated protein, required for epithelial morphogenesis. Delacour Delphine and Françoise Poirier

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Galectin-3 is a -galactoside binding protein widely expressed in all epithelia where it is involved in tissue homeostasis and cancer progression. We recently reported unique abnormalities in the identity of membrane domains in galectin-3 null mutant mice, suggesting that galectin-3 may participate in epithelial polarity program. We investigated the potential role of galectin-3 on early events in polarization of epithelial renal cells, using three-dimensional cultures of MDCK cells and also galectin-3 null mutant mouse kidneys. We show that depletion in galectin-3 systematically leads to severe perturbations of microtubular network associated with defects in membrane compartimentation, both in vitro and in vivo. Moreover, the absence of galectin-3 impinges on the morphology of the primary cilium, which is three times longer and unusually shaped. By immunological and biochemical approaches, we could demonstrate that endogenous galectin-3 is normally associated with basal bodies and centrosomes, where it closely interacts with core proteins, such as centrin-2. However, this association transiently occurs during the process of epithelial polarization. Interestingly, galectin-3 depleted cells contain numerous centrosome-like structures, demonstrating an unexpected function of this protein in the formation and/or stability of the centrosomes. Collectively, these data establish galectin-3 as a key determinant in epithelial morphogenesis via its effect on centrosome biology.

CHARACTERIZATION OF SNARE PROTEINS IN HUMAN NK CELLS

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NK cells participate in host protection by eliminating cells with altered expression of Major Histocompatibility Complex class I (MHC-I) molecules, which can result from viral infection or transformation. Even though their main cytotoxic mechanism is granule secretion, little is known about the components of the membrane fusion machinery that catalyze and regulate this kind of exocytosis. The present study explores, in human NK cells, the possible presence of one of the universal mediators of membrane fusion: the SNARE (soluble N-ethylmaleimide (NEM) sensitive factor attachment protein receptor) protein system, and examines its functional role in NK cell granule exocytosis. Up to now, we have identified numerous members of this protein family (as transcripts and as proteins) in the established cellular lines of NK cells (NK-92 and NK-L) as well as in peripheral blood human NK cells.
SNARE transcripts identified in human NK cells correspond with proteins previously identified as elements of the exocytic route in other cellular types of hematopoietic origin. Members of the three SNARE subfamilies have been identified: Syntaxin (2, 3, 4, 6, and 11), VAMP (2, 3, 7 y 8) and SNAP-25 subfamilies (SNAP-23). In addition, the expression of SNARE proteins was confirmed and their subcellular localizations were determined by confocal fluorescence microscopy. It widely emphasizes the coincident location of VAMP- 7 with cytotoxic granules, in accordance with previous studies of other authors, and as other SNAREs members are vesicles associated. In conclusion, human NK cells express several protein isoforms corresponding to the three SNARE families. This serves as starting point for identifying what SNARE proteins are forming the complexes that mediate the secretion of cytotoxic granules and to see if they are different from the SNARE complexes needed for cytokine secretion.
In order to evaluate the role of the identified SNARE proteins in granule exocytosis our current work focuses on optimizing inhibition assays.

Poster 26

Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions

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Dynamically polarized membrane proteins define different cell boundaries and have an important role in intercellular communication-a vital feature of multicellular development. Efflux carriers for the signaling molecule auxin from the PIN family are landmarks of cell polarity in plants and have a crucial involvement in auxin distribution-dependent development including embryo patterning, organogenesis and tropisms. Polar PIN localization determines the direction of intercellular auxin flow, yet the mechanisms generating PIN polarity remain unclear. Here we identify an endocytosis-dependent mechanism of PIN polarity generation and analyse its developmental implications. Real-time PIN tracking showed that after synthesis, PINs are initially delivered to the plasma membrane in a non-polar manner and their polarity is established by subsequent endocytic recycling. Interference with PIN endocytosis either by auxin or by manipulation of the Arabidopsis Rab5 GTPase pathway prevents PIN polarization. Failure of PIN polarization transiently alters asymmetric auxin distribution during embryogenesis and increases the local auxin response in apical embryo regions. This results in ectopic expression of auxin pathway-associated root-forming master regulators in embryonic leaves and promotes homeotic transformation of leaves to roots. Our results indicate a two-step mechanism for the generation of PIN polar localization and the essential role of endocytosis in this process. It also highlights the link between endocytosis-dependent polarity of individual cells and auxin distribution-dependent cell fate establishment for multicellular patterning.

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Poster 27

Polarized targeting of a G-protein activated potassium channel by a dileucine motif

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Inwardly rectifying potassium (K) channels activated by Gi/Go protein-coupled receptors (GIRK or Kir3.x) decrease membrane excitability by hyperpolarizing the membrane potential, slowing membrane depolarization, and shortening the action potential waveform. The molecular mechanisms underlying G protein regulation of GIRK channels have been extensively studied in the acetylcholine activated GIRK1/GIRK4 heteromultimer (IKACh) from atrial pacemaker cells and myocytes. In contrast to the widely characterized GIRKs from mammals, few functional studies exist of these channels in non-excitable cells where G-protein coupled receptors play a crucial role in cell development and differentiation. GIRK5 (Kir3.5) is an endogenous potassium channel from Xenopus laevis oocytes (Hedin et al 1996, Salvador et al 2001, Salvador et al 2003), a widely used functional expression system and studied model of meiotic maturation.

Specific lipid and protein distribution of the apical and basolateral membrane surfaces in epithelial cells are determined by a number of sorting and retention mechanisms; many of these are shared with other polarized cell types including neurons. Oocytes display a physical and molecular asymmetry; however no studies exist of maternal proteins trafficking in these cells. Endogenous phosphorylation of a unique tyrosine (Y16) at the amino terminus down-regulates GIRK5 in oocytes (Mora and Escobar, 2005). Therefore, we explored the role of other amino acid residues upstream of tyrosine Y16 by site directed mutagenesis, whole cell voltage-clamp recordings, immunoblot and confocal imaging. We identified targeting of GIRK5 to the vegetal cortex of the oocytes by a dileucine motif (EXXXLI). In conclusion, we determined for the first time the polarized trafficking of a maternal protein (GIRK5) in Xenopus laevis oocytes.

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Crosstalk between polarity proteins in cell polarization

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Cell polarity is essential for the development of multicellular organisms and many biological functions. Loss of epithelial cell polarity contributes to tumour invasion and metastasis, whereas efficient immune responses require proper polarization and migration of T-cells. Three complexes of conserved polarity proteins have been described to regulate various aspects of cell polarity, together with members of the Rho GTPase family. The Par3–aPKC–Par6 complex (Par complex), the Crumbs3–Pals1–PATJ complex (Crumbs3 complex), and the Scribble–Dlg–Lgl complex (Scribble complex) have been shown to regulate the establishment of cell polarity in various organisms. Our group has shown that the Rac activator Tiam1, in conjunction with the Par complex, is crucial for the establishment and maintenance of both apical-basal polarity of contacting epithelial cells as well as front-rear polarity of migrating epithelial cells. Furthermore, we have shown that Tiam1 and the Par complex are implicated in the establishment of Rap1- and chemokine-induced T-cell polarization as well as T-cell trafficking and trans-endothelial migration.
Although several molecular interactions between components of the three polarity proteins complexes have been described, little is known about the possible interplay and hierarchy between these conserved polarity protein complexes in these different polarization processes. Therefore, we are currently investigating the possible interconnection and interdependency of the Par, Scribble and Crumbs polarity complexes in different polarity models used in our lab, including epithelial apical basal polarity, polarized cell migration and T-cell polarity. Using RNA interference, chemical inhibitors and knockout cells to downregulate key molecules of the different complexes, we will monitor effects on polarization, localization and activation of the individual polarity proteins.
These experiments might uncover a potential function and hierarchy of the Par, Crumbs3 and Scribble complexes and will provide insight into the mechanism by which the individual proteins of the different polarity complexes cross-talk and are targeted to specific intercellular sites for correct polarization of cells in different cellular contexts. These findings on the function of polarity complexes in different cell types will help to understand the deregulation of polarity signalling pathways leading to pathological situations, including tumour formation, invasion and metastasis as well as defects of the immune system.

Poster 29

Function of the rab27a Munc13-4 interaction in Hematopoietic cells

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Regulated release of secretory lysosomes is a process used by NK cells and CTLs to eliminate infected and malignant cells. On the other hand, mast cells mediate inflammation through secretion of bioactive molecules from lysosomes. Because of the high lytic ability of the lysosomal content, secretion needs to be tightly regulated. Signaling pathways originating from activated immune receptors have been mapped to great detail, but how this is coupled to activation of the secretion machinery is poorly understood.

Rab27a controls regulated secretion in several cell types. It forms a ternary complex with melanophilin and unconventional myosins and functions in bringing melanosomes to the plasma membrane. However in hematopoietic cells a different effector of rab27a is present. Munc13-4 is a member of the Munc13 family; whose members are thought to perform a tethering role in synaptic vesicle release. Given the architectural parallels between the neuronal and immunological synapse, Munc13-4 likely serves a similar function in hematopoietic cells.

Munc13-4 and rab27a interact directly and specifically and both proteins are essential for regulated secretion of secretory lysosomes. It is not clear however, whether a complex between them is required for secretory lysosome release. Because Munc13-4 does not contain sequence that is homologous to the conserved rab27a binding region found in the melanocyte effectors, we generated a series of truncation mutants and tested them for rab27 binding. This yielded a stretch of 50 amino acids between the first C2 domain and the MUN domain. Subsequent site directed mutagenesis further refined the rab27 binding determinant to a patch of 6 amino acids in the context of full length Munc13-4. These binding mutants will provide a powerful tool to investigate the functional relevance of the interaction in living cells.

We are now in the process to analyze whether or not the mutants fail to rescue lysosome secretion. To this aim we use two different hematopoietic cell types namely the RBL-2H3 mast cell model and CTLs. Through a comparison of mast cells and CTLs we might be able to establish whether rab27a and Munc13-4 act through a conserved pathway. Endogenous rat Munc13-4 has either been silenced by siRNAs in the RBL-2H3 cells, or is not present because we employ CTLs isolated from FHL3 patients. Initial results suggest that the rab27a binding deficient Munc13-4 mutants appear to be partially mislocalized to endosomal structures in RBL-2H3 cells.

Genome-wide in vivo RNAi screen of conserved genes in Drosophila for regulators of epithelial cell polarity and tissue architecture

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What makes one tissue a different shape and form to another? One major factor is the morphology of the constituent cells themselves, however the genetic control of cell shape is poorly understood. To address this question we focus on the epithelial tissues of the fruit fly Drosophila. Epithelial tissues are made up of cells with a pronounced apical-basal polarity that are connected to each other by adherens junctions to form a sheet of cells. We wish to understand how epithelial cells establish and maintain this polarity, adhesion and shape. Understanding this is of direct relevance to cancer, because most human tumours are epithelial in origin and loss of epithelial morphology is a key step in tumour progression. For example, once tumour cells escape the epithelium, they can invade local tissues – a prerequisite for progression to metastasis.
To find novel genes regulating epithelial cell shape and polarity we have performed an in vivo genome-wide RNAi-based screen of conserved genes in the ovarian follicular epithelium of Drosophila melanogaster. This tissue was chosen as it constantly renews, allowing analysis of the full range of phases in epithelial differentiation. Furthermore, follicle cells stop proliferating during mid-oogenesis so cell competition is markedly reduced compared to the continuously dividing imaginal disc tissue. Consequently cells with polarity defects remain in the follicular epithelium and can easily be scored for a phenotype.
Each UAS-RNAi line was expressed in clones using a heat-shock inducible Gal4 driver (actin-flipout-Gal4) that is positively marked by UAS-GFP expression. Ovaries from these heat-shocked females were dissected and stained using DAPI and antibodies against an apical marker (atypical PKC) and a baso-lateral marker (Discs large), and processed for confocal microscopy. The GFP marker permitted the rapid identification of cells where the RNAi was expressed and the mosaicism of the Gal4 driver provided an internal adjacent control (i.e GFP-negative follicle cells). We then observed the localisation of polarity markers in epithelial cells where a certain gene has been knocked down and compared it to a control epithelial cell. We also monitored any changes in cell shape. We have discovered strong polarity phenotypes with some membrane trafficking genes and also genes of unknown function. Results of the screen will be presented.

SARA endosomes ensure the directional transport of Delta/Notch during asymmetric cell division

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Asymmetric cell division is important for the generation of different cell types during development as well as for the stem cell-dependent maintenance of adult tissues. We use the development of the mecanosensory bristles of the fruitfly, Drosophila melanogaster, to study the importance of endocytic trafficking for the signalling events that accompany asymmetric cell division. Sensory bristle development involves the asymmetric division of a Sensory Organ Precursor cell and the differential activation of Notch signalling in its two daughter cells. We have developed an assay that allows to visualize the endocytic trafficking of the endogenous populations of the ligand Delta and its receptor Notch in living tissue. We show that Delta / Notch internalized prior to division are transported to the posterior daughter cell in which Notch signalling is subsequently activated. The endosomes ensuring the asymmetric transport of Delta/Notch can be identified by the presence of the TGF-beta signalling adapter SARA. Mistargeting of SARA endosomes to the anterior daughter cell causes phenotypes indicative of ectopic Notch signalling. Notch activation involves the gamma-secretase-dependent release of the Notch Intra-Cellular Domain (NICD) which ultimately enters the nucleus and activates target genes. Interestingly we observe that NICD is present in SARA endosomes before, but not after SOP division. This disappearance of NICD from SARA endosomes requires the ligand Delta and gamma-secretase, in agreement with a potential scenario in which the final step of Notch activation would occur at the level of a subpopulation of multivesicular endosomes. Our observations suggest that asymmetric segregation of internalized Delta/Notch during SOP division contributes to the unequal activation of Notch signalling in the two daughter cells.

Coupling the division plane to cell polarity: phosphorylation of the NuMA-like protein LIN-5 in response to polarity signals

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Asymmetric division is essential during development for the generation of different cell types and maintenance of stem cell pools. During intrinsic asymmetric divisions, a cell first acquires polarity, then the mitotic spindle orients along this polarity axis and the cell divides. The resulting daughter cells inherit different cellular components and may thus acquire different fates. Although it is becoming clear how polarity cues are established during asymmetric cell division, it is unknown how these cues regulate the position of the mitotic spindle to determine the plane of division. A conserved complex of two G protein regulators, GPR-1 and GPR-2 , and a NuMA like protein LIN-5 acts downstream of polarity signals and controls the position of the mitotic spindle in asymmetric division. To understand how LIN-5 and GPR-1/2 are controlled by polarity signals during spindle positioning, our lab studies the post-translational regulation of LIN-5/GPR-1/2 complex formation and function. We found that LIN-5 is extensively phosphorylated in vivo, and are currently testing which candidate kinases are controlling these modifications by quantitative mass spectrometry. In addition, we examine which phosphorylation sites of LIN-5 are required for rescue of lin-5 null mutants. We have found that simultaneous mutation of two threonine residues in the N-terminus of LIN-5 completely abolishes lin-5 rescue. In the C-terminus of LIN-5, mutation of either of two serine residues results in milder cell division defects. Currently we address if and how asymmetric division and spindle positioning are affected in these mutants. This, together with the quantitative mass spectrometry experiments, will give us insights on how the division plane is controlled at the molecular level in asymmetric division.

The SH2-Domain Containing Inositol 5 Phosphatase 2 (SHIP2) is a Basolateral Determinant of Cell Polarity

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Establishment of epithelial polarity involves cell-cell and cell-extracellular matrix interactions as well as an important vesicle trafficking. Phospholipids are key element of membrane dynamics and vesicular trafficking, however, their involvement in epithelial cell polarity just started to be investigated. We demonstrated that Phosphatidylinositol (3,4,5) trisphosphate or PI(3,4,5)P3, specifically localized on the basolateral membrane is a key signal for polarization. We also show that PTEN localizes to the apical plasma membrane during epithelial morphogenesis to mediate the enrichment of PtdIns(4,5)P2 at this side.
Our current investigations demonstrated that the Src homology 2 (SH2) domain containing inositol-5-phosphatase 2 (SHIP2), another phosphatase which can hydrolyse [PI(3,4,5)P3] is also localized at the basolateral membrane of polarized MDCK cells. Knock down of SHIP2 using siRNA and dominant negative, induces disruption of cell polarity and cytokinesis defects. Our data also indicated that SHIP2 interacts with FAK and Ecadherin and regulates their cellular expression. We concluded that SHIP2 connects cell-cell and cell-extracellular matrix interactions signals to govern polarization. In addition, this work highlights a connection between the hScrib/dlg1 complex and the phosphoinositides metabolism.

Visualizing mRNA transport pathways

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Intracellular mRNA localization establishes protein asymmetries for polarization of somatic cells, oocytes, and embryos. In both invertebrates and vertebrates, mRNA localization during oogenesis generates asymmetric distributions of maternal determinants to establish the embryonic body plan. Localization of bicoid and nanos mRNAs to opposite ends of the Drosophila oocyte provides the sources for protein gradients that pattern the anterior-posterior body axis of the embryo. Posterior localization of nanos is also essential for its subsequent segregation to the germ cell precursors and ultimately for germline development. Later, during larval development, localization of nanos mRNA to dendrites of sensory neurons plays an important role in dendrite morphogenesis.

mRNA localization typically involves recognition of mRNA localization signals embedded in mRNAs by localization factors, packaging of mRNA-protein complexes into transport particles, transport of these particles, and finally anchoring at the target destination. We have developed a transgenic method for fluorescent tagging of mRNAs in vivo to investigate mechanisms of mRNA transport and anchoring. By visualizing movement of bicoid and nanos mRNAs in real time, we have shown that these mRNAs are localized concurrently during oogenesis by different localization pathways: bicoid transport is microtubule-dependent whereas nanos transport is diffusion based. In addition, these studies reveal temporal complexity of mRNA anchoring mechanisms.

Most recently, we have shown that unlike transport during oogenesis, segregation of nanos mRNA to the germ cells during embryogenesis occurs through dynein dependent transport on centrosome nucleated microtubules. Analysis of nanos mRNA particle motility in dendrites together with genetic dissection of factors required for its dendritic localization is also consistent with active transport of nanos. Surprisingly, posterior localization of nanos in oocytes, segregation of nanos to germ cells, and dendritic localization of nanos are all directed by the same cis-acting signal in the nanos 3' untranslated region. We are currently exploring this context dependence of mRNA transport.

Directed targeting into transcytotic pathways by surface modification of nanoparticles

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Brain tissue is difficult to target as the brain endothelial cells form a tight blood-brain barrier (BBB), protecting the brain from harmful xenobiotics, but also keeping potential medicines out. For drug delivery towards specific tissues in the human body, one may not target the cells within this tissue, but rather the tissue-specific endothelium that shields the tissue from the systemic circulation. In order to deliver drugs into the brain, we aim to exploit the brain's natural mechanisms for nutrient uptake. One of these mechanisms is transcytosis (endocytosis at the blood-side of the endothelium followed by exocytosis at the tissue-side), including receptor-mediated transcytosis and adsorptive endocytosis.
Previously, we have shown that the processing of nanoparticles via distinct endocytotic pathways is dependent on the size of the particles (Rejman et al. 2003). Particles of 100-200 nm were shown to be processed via clathrin-mediated endocytosis, whereas 500 nm particles were internalized via caveolae. Since brain endothelial cells are particularly rich in caveolae, we reason that brain caveolae may provide an efficient gateway to the brain.
Here, we study the uptake of 500 nm nanoparticles in the polarizing human brain endothelial cell line hCMEC-D3. The internalization of 500 nm nanoparticles by hCMEC-D3 cells is filipin-sensitive, and the particles colocalize with caveolin. To promote cellular binding of the nanoparticles and stimulate the process of transcytosis two strategies were followed:
I. to mediate receptor-mediated transcytosis the particles were coated with prion proteins (PrPc). Prion proteins are GPI-anchored proteins that are present in caveolae. They have been positively identified on hCMEC-D3 cells, and show homophilic interactions, that have been described to play a role in the transendothelial migration of monocytes (Viegas P. et al., 2006).
II. to mediate adsorptive transcytosis the surface of 500 nm nanoparticles was modified with excessive cationic charge by incorporation of a cationic polymer. Likewise, cationization of albumin is known to induce its transcytosis at the BBB.
We show that surface modifications of nanoparticles critically affects their interaction with and processing by cells.

An advection-triggered, self-organizing process for establishing PAR polarity domains in C. elegans

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Cell polarization describes a fundamental and persistent reorganization of cellular architecture in response to an asymmetric cue. This process often involves the formation of discrete membrane-associated “cortical” domains which possess specific components and acquire specialized functions thereby allowing localized differentiation of membrane compartments within a single cell. In complex eukaryotes, polarity domains are often associated with particular sets of PAR polarity proteins which serve as key integrators of upstream polarizing cues and downstream effector pathways. However, despite the importance of PAR domains in development, the mechanisms by which cells generate stable PAR domains remain unclear. In the nematode worm, C. elegans, PAR polarity is first established in the one-cell embryo and is essential for both the asymmetric first cell division and the asymmetric partitioning of cell fate determinants into the resulting daughter cells. In this process, the conserved complex of PAR-3, PAR-6 and aPKC define the anterior hemisphere of the embryo, while PAR-2 and the conserved kinase PAR-1 define the posterior. The distribution of the these two sets of PAR proteins appears mutually exclusive, an outcome which is thought to depend on mutual antagonism between the anterior and posterior PAR complexes. We have constructed a reaction-diffusion description of PAR polarity based primarily on two properties of PAR proteins: 1) that they diffuse on the membrane, and 2) that they displace one another from the membrane. We find that this simplified reaction-diffusion description of PAR proteins, upon incorporation of our quantitative estimates of PAR protein mobility in the embryo, is able to reproduce many of the key characteristics of PAR polarity in silico that we observe in live embryos. In our simulations, while we find that the basic mechanism of segregation is a result of the self-organizing properties of the PAR proteins, simple anteriorly directed cortical flow provides a robust polarization trigger. These simulations further suggest experimentally verifiable hypotheses for how the embryo controls the size and position of the anterior and posterior PAR polarity domains, several of which appear to be true in the embryo. This advection-triggered reaction-diffusion-driven process of PAR domain formation appears to provide a robust, inherently polarizable and highly flexible system for polarity establishment.

Poster 37

Apical sorting of Sonic Hedgehog in polarized MDCK cells: An indirect cholesterol-dependent pathway

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The morphogen Sonic Hedgehog (Shh), involved in the patterning of embryonic tissue and organs, is an interesting model protein to study certain aspects of the sorting mechanisms in polarized epithelial cells. It is the only known protein covalently modified by a cholesteryl group, which is added somewhere in the secretory pathway concomitantly with a proteolitic cleavage of the precursor protein. Similar to GPI-anchored proteins, Shh associates with lipid rafts but through its cholesteryl group. Epithelial cells address distinct proteins to their apical or basolateral domains through both exocytic and endocytic pathways. Although Shh is expressed in many epithelia during development and adulthood, its polarized sorting remains unclear and controversial. The evidence suggests apical or basolateral sorting depending on the model system examined. To get new insight into this problem, we transiently and stably expressed Shh in MDCK cells, in which the mechanisms for polarized protein sorting are better known. Pulse-chase experiments indicate that proteolysis (and cholesterol addition) occurs in the endoplasmic reticulum. In these cells, Shh associates with lipid rafts displaying different sensitivity to Lubrol and Triton X-100 extraction. At steady state, Shh localizes mainly to the apical surface, in congruency with the apical sorting role attributed to lipid rafts. Mutated, soluble Shh lacking the cholesterol adduct is secreted in a non-polarized fashion, indicating that cholesterol anchor conveys apical sorting information. However, biotinylation- and immunofluorescence-based cell surface targeting assays suggested that Shh apical sorting involves trafficking first to the basolateral surface and then transcytosis to the opposite pole. Besides the functional implications, these results can explain controversial previous observations (Financed in part by FONDAP grant# 13980001, a Basal Project from CONICYT, and a Millenium Project from Ministerio de Planificación, Gobierno de Chile).

Poster 38

How physical and geometrical constraints influence the organization of the microtubule cytoskeleton

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Microtubule-based structures that are essential to living cells are generated by multiple mechanisms. For instance, radial arrays called asters can be produced through cross-linking and moving microtubules by oligomeric motors. In vivo, this phenomenon occurs within the cellular boundaries, but how these boundaries affect the self-organizing process is not known. To approach this question, we studied the organization of microtubules inside droplets of eukaryotic cellular extracts with varying sizes and elastic properties. Our results show that the size of the droplet determined the final steady-state organization, which changed from symmetric asters to asymmetric semi-asters and finally to cortical bundles. A simple physical model recapitulated these results, identifying the main physical parameters of the transitions. The use of vesicles with more elastic boundaries resulted in very different morphologies of microtubule structures, such as asymmetric asters, "Y-branching", cortical, "rackets", and bundled organizations. Our results highlight the importance of taking into account the physical characteristics of the cellular confinement to understand the formation of cytoskeleton structures in vivo.

Poster 39

Contributions of hepatocyte polarity to a liver stage malaria infection

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Liver stage infection is the first obligatory step of Plasmodium, the causative agent of malaria, in the mammalian host. Motile sporozoites are deposited in the dermis during the bite of an infected mosquito, and migrate to the liver. After reaching the liver, a Plasmodium sporozoite will traverse several hepatocytes before invading a final one, inside a hepatocyte plasma membrane-derived parasitophorous vacuole. Once inside this protected compartment, the sporozoite will initiate a growth and replication cycle that will ultimately produce several thousand infectious merozoites. Upon egress from the host hepatocyte, the merozoites will initiate the symptomatic, blood stage phase of malaria. The basic biology that underlies the hepatocyte-Plasmodium interaction is not well understood, but the hepatocyte appears to provide a singular and unique niche suitable to allow complete parasite development. Hepatocytes are highly polarized epithelial cells, with basolateral (sinusoidal) and apical (canalicular) plasma membrane domains physically separated by tight junctions and maintained by polarized protein trafficking. During a recent siRNA screen of the human kinome carried out by Maria Mota's lab, several kinases with links to polarity were uncovered as modulators of liver stage infection. With these, as well as components of the tight junctional complex, which are known to be required for hepatits C virus infection of hepatocytes, as targets, we have initiated knockdown experiments with shRNAs to probe the requirement for these proteins during liver stage infection. Additionally, using GFP-expressing parasites and a variety of fluorescent markers of cellular architecture, we are investigating the trajectory and positional requirements of sporozoites during migration and invasion. To this end, we are carrying out live imaging studies in stably polarized (WIF-B9) and poorly polarized (Huh-7) hepatoma cells lines, as well as in mouse liver.

Poster 40

Cdc42-dependent remodeling of the cytoskeleton at the immune synapse is required for interferon-gamma secretion

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Regulated cytokine secretion by T lymphocytes is central in establishing an efficient immune response. Whereas much is known on the regulation of cytokine production, little is known on the regulation of cytokine exocytosis. We show here that inhibition of microtubule polymerization, which inhibits T cell polarization at the immune synapse, differentially affects the secretion of cytokines, i.e. it inhibits IFN-gamma secretion but not IL-2, CCL3 and CCL5 secretion. We also show that depletion of Cdc42, which disorganizes the microtubule and actin networks at the immunological synapse, inhibits IFN-gamma secretion without affecting IL-2 secretion. These data reveal a previously unidentified secretory pathway regulated by Cdc42-dependent remodeling of the cytoskeleton at the immune synapse that specifically controls IFN-gamma secretion.

The Synaptic Vesicle as a Prototypic Trafficking Organelle

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An extreme example of cell polarisation is found in the nervous system; neurones are highly polarized cells presenting two molecularly distinct compartments emerging from the cell body; a single axon and multiple dendrites. The correct specification and maintenance of axon and dendrites during neuronal development is an essential step underlying the proper transfer of information in the mature nervous system. Central to this polarisation lies the process of membrane trafficking. Membrane expansion at the growth cone requires the vectorial transport of membranes through the cell where they are added to the growing processes; while in mature neurones information is communicated by the release of neurotransmitters through the exocytosis and subsequent recycling of small synaptic vesicles in the axon terminal. Understanding the control of membrane trafficking is, therefore, critical to understanding the process of cell polarisation in the nervous system.

Synaptic vesicles have been intensely studied over the years, as they can be purified in large amounts and to a high degree of purity, making them amenable to sophisticated analytical methods. Using the full array of modern molecular, biophysical, electron microscopic and modelling techniques we investigated the protein and lipid composition of the synaptic vesicle. Here we present results from this systematic study, which illustrate the molecular constraints that govern the various functions a synaptic vesicle must perform; these data have already provided a useful reference for quantitative work on trafficking, docking and fusion.

As expected the composition of the synaptic vesicle was dominated by trafficking proteins, in particular the neuronal SNARE protein synaptobrevin 2, which is thought to be a key component of the membrane fusion machinery, along with its interacting partners syntaxin 1A and SNAP-25 (which reside in the plasma membrane). To investigate the regulation of the fusion apparatus we developed a novel in vitro fusion assay. Interestingly, our data show that synaptic vesicles are constitutively active fusion machines, dependent only on the activity of the SNARE protein synaptobrevin. This suggests that SNAREs operate as a ‘bare-bones’ fusion machinery in biological systems, without needing complicated docking complexes, priming reactions or activation steps. Instead fusion must be controlled by additional inhibitory factors acting as a break on this minimal machine. Given that synaptic vesicles are prototypic trafficking organelles, it is hoped that the principles elucidated using this assay will apply to other membrane trafficking processes.

Crosstalk between polarity proteins in cell polarization

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Cell polarity is essential for the development of multicellular organisms and many biological functions. Loss of epithelial cell polarity contributes to tumour invasion and metastasis, whereas efficient immune responses require proper polarization and migration of T-cells. Three complexes of conserved polarity proteins have been described to regulate various aspects of cell polarity, together with members of the Rho GTPase family. The Par3–aPKC–Par6 complex (Par complex), the Crumbs3–Pals1–PATJ complex (Crumbs3 complex), and the Scribble–Dlg–Lgl complex (Scribble complex) have been shown to regulate the establishment of cell polarity in various organisms. Our group has shown that the Rac activator Tiam1, in conjunction with the Par complex, is crucial for the establishment and maintenance of both apical-basal polarity of contacting epithelial cells as well as front-rear polarity of migrating epithelial cells. Furthermore, we have shown that Tiam1 and the Par complex are implicated in the establishment of Rap1- and chemokine-induced T-cell polarization as well as T-cell trafficking and trans-endothelial migration.

Although several molecular interactions between components of the three polarity proteins complexes have been described, little is known about the possible interplay and hierarchy between these conserved polarity protein complexes in these different polarization processes. Therefore, we are currently investigating the possible interconnection and interdependency of the Par, Scribble and Crumbs polarity complexes in different polarity models used in our lab, including epithelial apical basal polarity, polarized cell migration and T-cell polarity. Using RNA interference, chemical inhibitors and knockout cells to downregulate key molecules of the different complexes, we will monitor effects on polarization, localization and activation of the individual polarity proteins.

These experiments might uncover a potential function and hierarchy of the Par, Crumbs3 and Scribble complexes and will provide insight into the mechanism by which the individual proteins of the different polarity complexes cross-talk and are targeted to specific intercellular sites for correct polarization of cells in different cellular contexts. These findings on the function of polarity complexes in different cell types will help to understand the deregulation of polarity signalling pathways leading to pathological situations, including tumour formation, invasion and metastasis as well as defects of the immune system.

Poster 43

Mso1p, a novel phosphoinositide-binding protein interacts at sites of polarized exocytosis with the amino-terminal domain of Sec1p

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We have previously identified Mso1p as a Sec1-binding protein in yeast Saccharomyces cerevisiae. Sec1p family proteins are essential for membrane fusion. However, their mode of action and their functional regulation is still unclear. A short amino-terminal amino acid sequence in Mso1p is capable for the Sec1p binding both in vivo and in vitro. However, this N-terminal Sec1p-binding domain alone is not sufficient for Mso1p in vivo function. By using a novel transposone mutagenesis approach and site directed mutagenesis, we show that Mso1p interacts with the N-terminal domain of Sec1p. Neither Mso1p nor Sec1p contain obvious membrane targeting motifs. However, using the Bimolecular Fluorescence Complementation technique (BiFC), we show that they interact at the plasma membrane at the sites of polarized secretion. This interaction or its site is affected by defective small GTPase Sec4, its GEF Sec2, t-SNARE Sso2 and Sec18. MSO1 deletion is synthetically lethal with the phosphatidylinositol 4-monophosphate (PI4P) 5-kinase mss4. We now show that Mso1p is a phosphoinositide-binding protein and that this binding enhances Mso1p membrane interaction. Mso1p displays low sequence homology with the phosphoinositide-binding domain of neuronal Sec1-binding Mint1 proteins that regulate neuronal exocytosis. Our results suggest that Mso1p represents the functional homologue of Mint proteins in exocytotic membrane fusion in yeast. These results suggest a novel functional conservation within eukaryotic membrane fusion and a central regulatory role for Mso1p in connection with Sec1p in this process.

The Specificity of COPII Cargo Selection in the ER Export of Vangl2 for Neural Tube Development

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The objective of this study was to determine the role of a specific COPII component, Sec24B, in the establishment of planar cell polarity and thus the development of the neural tube. A prior murine forward genetics screen for neural tube defects found a premature stop codon in this protein, a component of the COPII budding complex responsible for the transport of cargo proteins out of the endoplasmic reticulum (ER). Using immunoprecipitation and localization, we have shown that this premature stop codon at Tyr653 renders Sec24B non-functional. Because neural tube development depends on planar cell polarity, we set out to identify what membrane components of the planar cell polarity pathway might depend on Sec24B for their exit from the ER. Through the use of an in vitro COPII budding reaction, we were able to demonstrate that the ER export of Vangl2 is specifically dependant on Sec24B. In addition, we have determined that commonly studied Looptail point mutations in the C-terminal cytoplasmic domain of Vangl2 block its exit from the ER in vivo and in vitro. We conclude that Sec24B has a unique role among its paralogs in the ability to transport specific cargo molecules, like Vangl2, and that the efficient ER export of membrane proteins implicated in planar cell polarity is required for proper neural tube development.

Tube formation by complex cellular processes in Ciona intestinalis notochord

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In the course of embryogenesis multicellular structures and organs are assembled from constituent cells. One structural component common to many organs is the tube, which consists most simply of a luminal space surrounded by a single layer of epithelial cells. The notochord of ascidian Ciona forms a tube consisting of only 40 cells, and serves as a hydrostatic “skeleton” essential for swimming. While the early processes of convergent extension in ascidian notochord development have been extensively studied, the later phases of development, which include lumen formation, have not been well characterized. Here we used molecular markers and confocal imaging to describe tubulogenesis in the developing Ciona notochord. We found that during tubulogenesis each notochord cell established de novo apical domains, and underwent a mesenchymal-epithelial transition to become an unusual epithelial cell with two opposing apical domains. Concomitantly, extracellular luminal matrix was produced and deposited between notochord cells. Subsequently, each notochord cell simultaneously executed two types of crawling movements bi-directionally along the anterior/posterior axis on the inner surface of notochordal sheath. Lamellipodia-like protrusions resulted in cell lengthening along the anterior/posterior axis, while the retraction of trailing edges of the same cell led to the merging of the two apical domains. As a result, the notochord cells acquired endothelial-like shape and formed the wall of the central lumen. Inhibition of actin polymerization prevented the cell movement and tube formation. Ciona notochord tube formation utilized an assortment of common and fundamental cellular processes including cell shape change, apical membrane biogenesis, cell/cell adhesion remodeling, dynamic cell crawling, and lumen matrix secretion.

Syndecan-4 regulates the Rac1 activity and influences the localization of Par6 subsequently mediating epithelial-mesenchymal transition

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Rho family of small GTPases constituted by Rac, Rho and Cdc42 plays central role in determination of cell morphology controlling diverse biological processes such as cell adhesion, migration, polarity and differentiation; therefore their specific regulation is very important. We have found that syndecan-4 was able to modulate the activity of Rac1 exclusively, and determine subsequently an epithelial-mesenchymal transition (EMT) in MCF-7 breast adenocarcinoma cells. Syndecan-4 belongs to the type I transmembrane proteoglycans bearing heparan-sulfate chains on its extracellular segment. Among the syndecans only syndecan-4 is expressed ubiquitously, required in the establishment of focal adhesions and involved in the organization of the actin cytoskeleton.
We observed that the phenotype of the MCF-7, HEK297 cell lines was changed depending on the mutations of the cytoplasmic serine of syndecan-4, which is target of phosphorylation. The phosphomimetic Ser179Glu mutation induced migratory mesenchymal phenotype while the phospho-resistant Ser179Ala mutation enhanced the epithelial polarization. Based on these results our aim was to reveal the biochemical background of the phenotypical changes. It was realized that syndecan-4 can regulate the Rac1 activity in a phosphorylation-dependent manner, that is, the phospho-resistant form elevated and the phosphomimetic form down-regulated the level of the Rac1-GTP. Examining the inhibitory effect of phospho-syndecan-4, it was unraveled that the interaction of Rac1 and Tiam-1, a Rac-GEF, was restricted.
During the epithelial polarization the adherens actin belts appeared and the height of cells was raised. On the contrary in migratory cells stress fibers were reorganized. The administration of PMA reversed the phenotypes: suppressing the migratory phenotype of the phosphomimetic mutation of syndecan-4 favoring mesenchymal-epithelial transition, stimulating solitary cell migration in wild type MCF-7 cells and collective migration of cell clusters in cells expressing the phospho-resistant mutant. In accordance with these, the level of the active Rac1 was changed: increased in the Glu mutant and decreased in the Ala mutant syndecan-4 expressing cells upon PMA treatment. Furthermore, syndecan-4 was found to be associated to Par6, a member of the PAR polarity complex. In vivo immunofluorescent microscopy revealed that the Ser179Ala syndecan-4 was observed in the apico-lateral surface, on the contrary, the Ser179Glu syndecan-4 was accumulated in the trailing edges of migratory cells co-distributing with Par6 all the time.
Overall, we suggest that syndecan-4 can modulate Rac-1 activity in a phosphorylation-dependent manner contributing to an active Rac1 gradient in apico-basal and front-rear orientations and determining the localization of Par6.

Class I Rab11-FIPs are effector targets for the Rab14 GTPase

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*Eoin E. Kelly*Rab11 and Rab14 are two related Rab GTPases that are known to function in endosomal recycling and Golgi to early/sorting endosome transport processes. We, and others, have identified a group of proteins that interact with Rab11, known as the Rab11-Family Interacting Proteins (FIPs), and function as Rab11 effectors. These FIP proteins have been sub-classified into two groups - class I (FIP2, RCP and Rip11) and class II (FIP3 and FIP4). Here, we identify the class I FIPs as dual Rab effectors by demonstrating that they also bind Rab14 in a GTP-dependent manner. We show that these interactions are specific for the class I FIPs and that they occur at their carboxy-terminal region, which encompasses the previously described Rab11-Binding Domain (RBD). Furthermore, we show that Rab14 significantly co-localises with transferrin receptor (TfnR)-positive endosomes and with the class I FIPs on the endosomal-recycling compartment (ERC) during interphase; and that during cytokinesis Rab14 localises to the cleavage furrow/midbody. The data presented here, which identifies the class I FIPs as the first effector proteins for the Rab14 GTPase, indicates greater complexity in the Rab-binding specificity of the class I FIP proteins.

Asymmetric cell division during pancreas development

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Asymmetric cell division is an important process for proper development of early embryo and organogenesis. In mouse embryo, brain and skin are the only organs known to be developed through asymmetric division of epithelial progenitors. Pancreas is an endoderm-derived organ comprised of endocrine, exocrine, and ductal epithelial cells. During pancreatic development, both endocrine and exocrine cells differentiate from ductal epithelial progenitors. Upon their differentiation, endocrine cells migrate from the epithelial progenitor layer and cluster into islet of Langerhans. Since epithelial progenitor cells in the developing pancreas have an apical-basal polarity, we hypothesize that endocrine cells may arise through asymmetric cell division of progenitors. To determine if asymmetric cell division occurs during pancreatic endocrine cell differentiation, we are in the process of measuring division axes of epithelial progenitors undergoing cell division at e14.5, when endocrine cells differentiate in mouse embryos. To lay the ground to the functional assessment of molecular determinants, we are also determining the expression pattern of asymmetric cell division markers, including par3, inscuteable, lgn and ags3 in e14.5 mouse pancreas. Through our studies, we will determine if cell diversity in the pancreas is controlled by the asymmetric inheritance of specific molecular cues and whether the 3D geometry of progenitors in the epithelium is used to position these cues.

Coordinated regulation of apical junctional complexes

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Polarized epithelia exhibit a distinct morphology characterized by discrete apical and basolateral membrane domains. Different functional activities partition to each of these membrane domains with the formation of apical junctional complexes participating in the generation of epithelial polarity. These apical junctional complexes contain both cadherin-based adherens junctions and tight-junctions. Collectively, these complexes provide both a barrier to paracellular transport and a platform to translate signals to the cytoskeletal network. The ability of apical junctional complexes to rapidly reorganize the cytoskeleton is a requirement in many morphological processes and functional relationships exist between the different junctional components to coordinate these rearrangements. One important interrelationship is a requirement for E-cadherin-mediated cell-cell adhesion to support the formation of tight junctions. It has been shown that many proteins having the capability to interact with the cytoskeleton are found at both cadherin-based and tight junctions. Using a knockdown and rescue approach, we show that N-WASP is required for the integrity of both E-cadherin cell-cell contacts, and for the localization of tight junction proteins in epithelial monolayers. An inability of N-WASP knockdown cells to organize tight junction proteins is apparent in 3D cyst cultures and the central lumen fails to form correctly. We show that N-WASP, through the action of the Arp2/3 actin nucleation complex, may provide the molecular basis for the coordination of junctional assembly and disassembly during epithelial morphogenesis.

A dynamic model for the morphogenesis of the Golgi apparatus

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While there has been considerable progress in understanding the molecular biology of the secretory pathway of mammalian cells, the fundamental question how the most prominent and complex organelle of the pathway, the Golgi apparatus, is formed and maintained has remained
largely elusive. Using a minimal self-organizing scheme based on incoming transport from the nearby endoplasmic reticulum and aging of Golgi fragments (’cisternal maturation’), we are able to explain the de novo formation of a Golgi apparatus. Moreover, we can derive a phase space which secretion rates support the formation of a proper stack of Golgi cisternae. Our simulations are consistent with analytical considerations and agree well with existing experimental data.

Characterizing the cellular role of Anthrax toxin receptors

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Anthrax toxin is an AB-type toxin produced by Bacillus anthracis and is thought to play a crucial role in the anthrax pathogenesis and impairment of the host immune machinery. Anthrax toxin is composed of three independent polypeptide chains: the lethal factor (LF), the edema factor (EF) and the protective antigen (PA) . LF is a zinc dependent metalloprotease that cleaves all MAP kinase kinases with the exception of MKK5 . EF is a calmodulin dependent adenylate cyclase that is responsible for the edema observed in anthrax patients . PA has no enzymatic activity and is involved in escorting EF and LF to the cytoplasm. Cellular entry of the toxin is mediated by the Anthrax toxin receptors (ATR) present at the cell surface. Two receptors have so far been identified: Tumor Endothelial Marker (TEM8) and Capillary morphogenesis gene 2 (CMG2). The cellular roles of these receptors have been rather unclear. To characterize the cellular role of ATRs, we performed a screen using membrane yeast 2-hybrid system. This system is based on the reconstitution of ubiquitin from its N-terminal and C-terminal halves, the latter of which is fused to a reporter protein that is released upon prey-bait interaction. Since the ubiquitin reconstitution occurs in the cytoplasm, and not in the nucleus, as is the case for reconstitution in classical yeast 2-hybrid systems, it can be used for membrane proteins. These screens have led to significant number of hits. We are now performing the validation of these targets in mammalian cells and more specifically in the epithelial cells and the skin cells which are polarized cells. A detailed study os these partners would enable us in understanding and implicating the ATRs in different cellular pathways.

Role of the apical Par-aPKC complex in the imaginal disc

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The aim of the present work is to study the regulation and function of the polarity determinant DaPKC in the epithelial cells of the imaginal discs of Drosophila. Our analysis of the subcellular distribution of the different polarity determinants in the wing disc epithelium has revealed subtle differences to their localization in the embryonic epithelium. Thus, whereas apical DaPKC, Crumbs (Crb) and Echinoid (Ed) and basolateral Discs large (Dlg) and Scrible (Scrib) maintain their non-overlapping subcellular distributions, PATJ and Crb overlap with DaPKC but also extend further apically and basally, respectively. In the discs, proper activity of polarity proteins, tissue organization and growth are closely related. Thus, loss of function of basolateral polarity markers such as Dlg), Lethal giant larvae (Lgl) or Scrib causes strong tissue overgrowth. We have found that clones of DaPKC null cells are scarcely viable and are extruded from the epithelium, accumulating high levels of actin and showing delocalization of Armadillo, D-E cadherin and Ed, indicative of compromised adhesion. In addition, Crb is lost from the membrane although distribution of Dlg and Scrb is not modified. Over-expression of membrane targeted DaPKC (UAS-DaPKCCAAXWT) in the wing disc causes depletion of Ed or Arm and tissue overgrowth. This overgrowth is similar to that found in scrb/lgl/dlg mutant discs, reinforcing the proposal of functional antagonism, described in the embryo, between apical and basolateral determinants. However, DaPKCCAAXWT expressing clones cause overgrowth whereas clones of cells mutant for basolateral identity determining genes fail to grow and are eliminated from the disc epithelium. Constitutive activation of DaPKC is also associated with trafficking defects in the wing disc epithelium. Thus, DaPKCCAAXWT over-expressing cells show enlarged accumulation of the early endosomal marker Hrs and abnormal dextrane uptake. We are analyzing to what extent defective trafficking affects the signalling pathways that operate in the wing disc and their contribution to the mutant phenotype. We propose DaPKC to behave as a protooncogene in Drosophila, as it has been shown in vertebrates, which should play a key role in the regulation of cell proliferation and in the maintenance of epithelial integrity in the developing wing disc.

CDC42-dependent MTOC polarization is required Antigen Trafficking and Presentation in B lymphocytes

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B-lymphocytes are professional antigen presenting cells, which efficiently capture Antigens (Ag) and process them for presentation onto MHC class-II molecules to CD4+ T cells. Ag capture by B lymphocytes is mediated by their surface antigen receptor, the B cell receptor (BCR). The BCR must ensure coordinated transport of MHC class II- and Ag- containing vesicles for them to converge in compartments devoted to Ag processing. Here we show that BCR engagement in primary B cells triggers polarized vesicular trafficking of MHC class II-containing lysosomes towards the Microtubule Organizing Center (MTOC). This process relies on the small GTPase CDC42, which (1) re-orientates the microtubule network of BCR-stimulated cells towards the site of Ag internalization and (2) couples the transport of MHC II-carrying lysosomes to this polarization event. Impairment of MTOC and lysosome polarized transport compromises the Ag processing capacity of B cells. Therefore, CDC42-dependent B lymphocyte polarization is essential for Ag processing and presentation.

The transcription factor ZONAB is a master switch between proliferation and apical differentiation in kidney proximal tubular cells (PTC.

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A central feature of embryogenesis and tissue repair is the switch between proliferation and differentiation. The transcription factor, ZONAB, reported to translocate into nuclei to promote cyclin D1/PCNA expression and cell proliferation, or to be sequestered by ZO-1 at tight junctions, qualifies as a candidate polarity regulator. We have investigated the expression and effects of ZONAB in three complementary models: kidney cancer, kidney development and in vitro polarization of OK cells, a well-established proximal tubular cell (PTC) line. In silico analysis of clear cell renal carcinoma microarray databases and quantitative RT-PCR on additional samples systematically disclosed increased ZONAB expression and proliferation markers, inversely correlating with expression of differentiation markers (the tandem apical endocytic receptors, megalin and cubilin, and villin). During ontogeny, E13.5 mice embryos showed a strong ZONAB labelling by in situ hybridization in kidney cortical primary tubules. Expression profiles for ZONAB mRNA and protein during further kidney development revealed that disappearance of ZONAB and PCNA was concomitant with appearance and increased expression of megalin and cubilin. The same opposite expression profile was observed upon in vitro polarization of OK cells and was functionally reflected by enhanced apical receptor-mediated endocytosis of megalin and cubilin ligands. To address if the opposite regulation between ZONAB and endocytic receptors was a cell-autonomous response that could be distinguished between adjacent cells, OK cells were sparsely plated to form small colonies, where junctional belts are continuous only around central cells but are interrupted at the outer margin of peripheral cells. The latter showed strong ZONAB nuclear staining and entry into S-phase (EdU incorporation) but minimal megalin expression, while neighbouring central cells were devoid of nuclear ZONAB, did not enter S phase and abundantly expressed megalin. Transient transfection with ZONAB repressed megalin and cubilin promotors in luciferase reporter assays, which was reversed by ZO-1 co-transfection, demonstrating the specificity of the interaction. Binding of ZONAB to endogenous megalin and cubilin promoters was confirmed by chromatin immunoprecipitation. Stable ZONAB transfection strongly repressed megalin and cubilin expression and endocytic function. ZONAB overexpression also repressed villin expression and alpha-tubulin acetylation, as reflected by impaired maturation of the brush border and the primary cilium. Interestingly, polarity down-regulated ZONAB transcription and induced its proteasomal degradation. We propose that ZONAB is a master transcription factor, acting as a switch between proliferation and differentiation via direct and simultaneous opposite effects, and down-regulated by polarity at transcriptional and post-translational levels.

Characterization of the MAL2 positive compartment in oligodendrocytes

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Oligodendrocytes, the myelin-producing cells of the central nervous system, segregate different surface subdomains at the plasma membrane as do other differentiated cells, such as polarized epithelia and neurons. To generate the complex membrane system that characterizes myelinating oligodendrocytes, large amounts of membrane proteins and lipids need to be synthesized and correctly targeted. In polarized epithelia, a considerable fraction of apical proteins are transported by an indirect pathway involving a detour to the basolateral membrane before being internalized and transported across the cell to the apical membrane by a process known as transcytosis. The Apical Recycling Endosome (ARE) or the Subapical Compartment (SAC) of hepatocytes, are intracellular trafficking stations involved in the transcytotic pathway. MAL2, an essential component of the machinery for basolateral-to-apical transcytosis, is an ARE/SAC resident protein. Although MAL2 expression has been detected in the KG-1C oligodendroglial cell line (Bello-Morales et al., 2005), no studies on MAL2 distribution has been reported in these cells yet. We are investigating the expression of MAL2 in oligodendrocytic cell lines, as well as its regulation during cell differentiation. In addition, the characteristics of the MAL2 positive compartment of differentiated cells and its similarity with the ARE/SAC of epithelial cell lines have been studied. We suggest that the MAL2 positive compartment in oligodendrocytic cells could be a structure analogous to the ARE/SAC and might have an important role in the sorting of proteins and lipids for myelin assembly during oligodendrocytes differentiation.

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Afferent Neurons of the Zebrafish Lateral Line Are Strict Selectors of Hair-Cell Orientation

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Hair cells in the inner ear display a characteristic polarization of their apical stereocilia across the plane of the sensory epithelium. This planar orientation allows coherent transduction of mechanical stimuli because the axis of morphological polarity of the stereocilia corresponds to the direction of excitability of the hair cells. Neuromasts of the lateral line in fishes and amphibians form two intermingled populations of hair cells oriented at 180° relative to each other, however, creating a stimulus-polarity ambiguity. Therefore, it is unknown how these animals resolve the vectorial component of a mechanical stimulus. Using genetic mosaics and live imaging in transgenic zebrafish to visualize hair cells and neurons at single-cell resolution, we show that lateral-line afferents can recognize the planar polarization of hair cells. Each neuron forms synapses with hair cells of identical orientation to divide the neuromast into functional planar-polarity compartments. We also show that afferent neurons are strict selectors of polarity that can re-establish synapses with identically oriented targets during hair-cell regeneration. Our results provide the anatomical bases for the physiological models of signal-polarity resolution by the lateral line.

Regulation of the assembly of the tight junction by the ubiquitin-proteasome system

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The program of epithelial cell polarity development is triggered by the engagement of two types of adhesion molecules: integrin and E-cadherin. We have reported a role for the small G protein Arf6 and its exchange factor EFA6 (Exchange Factor for Arf6) in the assembly of the apical actin ring onto which the tight junction (TJ) is anchored in response to E-cadherin engagement in MDCK cells. Of particular intesrest, we observed that the levels of EFA6 are up-regulated before the formation of the TJ and return to normal levels when the cell established its tight junction. Overexpression of EFA6, that mimicks the one induced by E-cadherin engagement, leads to the accelerated reorganization of the apical actin cytoskeleton ring onto which the TJ is anchored. Conversely, depletion of EFA6 by RNA interference impairs the assembly of the TJ. We have found that EFA6 is poly-ubiquitinated following E-cadherin engagement. This ubiquitination is transient and preceeds the decrease of EFA6 expression occuring during cell polarity development. Low concentrations of proteasome inhibitors increase EFA6 expression and mimic the morphogenic and functional effects of EFA6 overexpression on TJ formation. We have identified an E3-ligase and an ubiquitin protease that bind to EFA6 and control its ubiquitination status during cell polarity development. We will prensent our latest results regarding the spatio-temporal distribution and activities of both enzymes relative to EFA6, the actin cystokeleton remodelling and TJ assembly. Our studies suggest a molecular mechanism by which the coordinate activities and cell distribution of both ubiquitin modifying enzymes control a signaling pathway downstream of the E-cadherin that includes EFA6, Arf6 activation, the actin cytsokelton remodelling and the TJ assembly in polarizing epithelial cells.

Poster 58

Regulation of lumen formation and transcytotic transport by a novel member of the formin family in hepatoma HepG2 cells

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In epithelial cells, proteins destined for the apical surface travel directly from the Golgi or indirectly by making a detour to the basolateral surface before being internalized and transported to the apical membrane in specialized endosome carriers in a process known as transcytosis. Hepatocytes constitute a simple model for apical transport studies as the indirect route constitutes the sole mechanism for apical targeting of proteins attached to the membrane by a single anchor. MAL2, an integral membrane protein of the MAL family, is an essential element of the machinery for basolateral-to-apical transcytosis in hepatoma HepG2 cells (de Marco et al., 2002). At steady state, MAL2 predominantly distributes at the apical surface but inhibition of transcytosis in MAL2-silenced cells does not produce cargo arrest early in the transcytotic pathway. Analysis of MAL dynamics revealed that a fraction of apical MAL2 redistributes into peripheral endosome elements to concentrate cargo internalized from the baolateral membrane. Then, these MAL2+ endosomes progressively fuse and move towards the apical surface for cargo delivery (de Marco et al., 2006). MAL2 dynamics, therefore, explains the apparent paradox whereby MAL2 silencing results in the accumulation of cargo in endosome elements distant from the apical surface as if MAL2 were mediating transcytosis at a distance.

Diaphanous-related formins (Drfs) bridge G-protein signals and the actin and tubulin cytoskeletons via their ability to bind active small Rho GTPases. Unlike actin-related protein 2/3 (Arp2/3) complex that generates branched actin filaments, Drfs are involved in formation of linear actin filaments. Drfs have been found to modulate a number of intracellular processes such as endosome motility, microtubule stabilization and cytokinesis.

To obtain insights into the mechanism of transcytosis, we have carried out a search for proteins that interact with MAL2 and identified Drf5, a novel formin with a Drf domain organization, as a MAL2 partner. MAL2 and a fraction of Drf5 colocalized at the apical surface of HepG2 cells. Silencing of Drf5 decreased MAL2 dynamics, slowed down transcytotic transport and diminished the number of intercellular lumens. Drf5, therefore, controls lumen formation and apical transcytosis by regulating MAL2 trafficking in HepG2 cells.

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Cell-to-cell trafficking via the ER-membrane in plants

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It was a surprise for cell biologists when it turned out that a wide range of macromolecules including soluble proteins are able to move from cell to cell in plants. The transport takes place via plasmodesmata, which are analogous to animal cell's gap junctions. The latter do not traffic larger molecules, however, recent evidence suggests that so-called tunnelling nanotubes exist in animal cells allowing trafficking of macromolecules and even organelles from cell to cell. These structures may reveal the presence of a universal inter-connectivity in tissues.

For plant cells an important role of the endoplasmic reticulum (ER) in cell-to-cell trafficking is beginning to emerge. Some virus movement proteins, which are by far too large to pass a plasmodesma, are thought to traffic through the cytoplasmic channel in an unfolded form while others may pass along the endoplasmic reticulum. The ER is a series of membrane bound cisternae that are primarily responsible for manufacturing and delivery of proteins to various locations within the cell. Furthermore, ER is a main component in a plasmodesma where it forms a thin cylinder offering a second intercellular pathway. Cytoskeleton elements, actin and myosin, are believed to be important for the maintenance of the physical size of plasmodesmata, and they may also play a role in intercellular transport of macromolecules, like their transport-role inside the cell. However, the mechanisms by which macromolecules move are still speculative and whether also membrane proteins are able to move is largely unknown.

An outstanding example for intercellular transport occurs in the sugar-transporting vascular tissue. We have shown that the function of sucrose transporters is not merely uptake of sucrose from photosynthesizing leaves, but that it has an overall retrieval function in transport tissue. However, it is a cell biological enigma why this sucrose transporter in some plant families is localised to the sieve elements, which are unable of protein synthesis, and not to the neighbouring companion cells where its transcription takes place. This spatial separation raises the question of intercellular protein trafficking.

Our working hypothesis is that membrane proteins traffic via the ER-membrane itself. Recent data, using animal cells, has shown that the dynamic ER membrane network is in constant contact to the plasma membrane and that local attachments of the two membrane systems enable a direct communication of ER- and plasma membrane-anchored proteins. We have imaged similar points of attachment in plant cells. A long-term goal of our group is to unravel whether and how membrane proteins can move from cell-to-cell in plants using high-resolution molecular imaging.

Ojoplano-mediated basal constriction is essential for optic cup morphogenesis

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Although the vertebrate retina is a well-studied paradigm for organogenesis, the morphogenetic mechanisms that carve the architecture of the vertebrate optic cup remain largely unknown. Understanding how the hemispheric shape of an eye is formed requires addressing the fundamental problem of how individual cell behaviour is coordinated to direct epithelial morphogenesis. We have analyzed the role of ojoplano (opo), an uncharacterized gene whose human ortholog is associated with orofacial clefting syndrome, in the morphogenesis of epithelial tissues. Most notably, when opo is mutated in medakafish, optic cup folding is impaired. We characterize optic cup morphogenesis in vivo and determine at the cellular level how opo affects this process. Opo encodes a developmentally regulated transmembrane protein that localizes to compartments of the secretory pathway and basal end-feet of the neuroepithelial precursors. We show that Opo regulates the polarized localization of focal adhesion components to the basal cell surface. We propose a model of retinal morphogenesis whereby basal focal contacts are required to generate mechanical tensions. These, transmitted by individual cells, drive in turn the macroscopic folding of the vertebrate optic cup.

This novel gene will serve now as an anchoring point to unveil potential partners that may also participate in the molecular machinery involved in optic cup morphogenesis

Crumbs 3A polarity complex: new partners involved in polarized cell migration

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The crumbs 3A complex is involved in the establishment and maintenance of apical domain of epithelial cells. It is composed of Crb3A, Pals1, PATJ and Par6 proteins and we have identified Hook2 (Hk2) and Mupp1, as new interactors by GST-pulldown assays with Crb3A cytoplasmic tail. Hk2 is a microtubule-binding protein associated with Golgi apparatus and we show that it is necessary for its correct positioning during polarized cell migration. Interestingly, we also have shown that Hook2 C-terminal domain binds to Par6 indicating that it might play a role as an adaptor between Par6/aPKC and Crb3A complexes. Mupp1 is structurally very similar to Patj; both proteins have multiple protein-protein interaction domains: a L27 domain and 8 to 13 PDZ domains. We showed that Mupp1 interacts with Pals1 but not with Patj, suggesting the existence of another complex composed of Crb3A/Pals1/Mupp1 in epithelial cells. Using RNA interference we showed that Mupp1, Pals1 and Patj are co-regulated and co-stabilized in the cell. Down-regulation of Pals1 induces both a loss of Patj and Mupp1. The mechanisms underlying this regulation are under investigation. Loss of Mupp1 affects Golgi reorientation but, as opposed to Patj, not migration in mammary epithelial cells by using a wound-induced migration assay. Together our data indicate that Patj and Mupp1 belong to two structurally independent but functionally linked complexes with different downstream effectors in cell migration events. These findings reveal novel roles for Crb3A complex in Golgi apparatus reorientation in polarized cell migration.

RhoGTPase RhoBTB2 and the regulation of cell polarity in breast cancer

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RhoGTPases are involved in a wide variety of cellular processes. We have recently been characterising the novel RhoGTPase, RhoBTB2. RhoBTB2 has previously been reported to be a tumour suppressor, with expression silenced or decreased in a variety of cancers. We used siRNA technology, followed by microarray analysis, to mimic the loss of RhoBTB2 expression in lung cancers. Subsequent studies, in both head and neck squamous cell carcinoma (HNSCC) cells and primary keratinocyte cells, led us to a chemokine, CXCL14, which was found to be decreased following RhoBTB2 silencing. Overexpression of RhoBTB2 using a lentivirus, caused the re-instation of CXCL14 expression and secretion.

CXCL14 has previously been shown to be expressed within epithelial cells of the mammary duct in normal circumstances. However, as tumourigenesis proceeds, CXCL14 is lost from the epithelial cells but starts to be expressed in the surrounding myoepithelial cells. We postulate that this is accompanied with a change from autocrine to paracrine function of CXCL14.

Mammary S1-HMT cells have been used successfully in many studies to determine the effect of various factors on the formation of mammary ducts. These cells, when seeded onto a layer of Matrigel, naturally form ducts and the accompanying mammary lumen. A loss of polarity within the cells that form these mammary ducts can have substantial implications for correct duct formation. If the cyst fails to form precisely as required, the protective layer which keeps cells within the duct disintegrates. If this occurs, the movement of tumour cells becomes less restricted. This, in parallel with a chemotactic effect of CXCL14, would therefore allow invasiveness to increase. This study is of importance in explaining changes between Ductal carcinoma in situ (DCIS) and invasive breast cancer.

Identification of a low energy epithelial polarity pathway

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We identified mutations in the single Drosophila melanogaster AMPK catalytic subunit AMPKalpha. Surprisingly, ampkalpha mutant epithelial cells lose their polarity and overproliferate under energetic stress. LKB1 is required in vivo for AMPK activation, and lkb1 mutations cause similar energetic stress-dependent phenotypes to ampkalpha mutations. Furthermore, lkb1 phenotypes are rescued by a phosphomimetic version of AMPKalpha. Thus, LKB1 signals through AMPK to coordinate epithelial polarity and proliferation with cellular energy status, and this might underlie the tumor suppressor function of LKB1 (Mirouse et al, J Cell Biol 2007).
Moreover, Lee et al (Nature, 2007) showed that AMPK is a Myosin II kinase in vitro and in vivo and that this function is sufficient to explain its role in epithelial polarity since a phosphomimetic form of the Myosin II rescues the polarity defect due to the loss of function of AMPK. This last point also indicates that there is no requirement for a localized control of Myosin II phosphorylation during epithelial polarization, suggesting that it might be important to understand how the Myosin II itself is localized.
Dystroglycan localizes to the basal domain of epithelial cells and has been reported to play a role in apical-basal polarity. We showed that Dystroglycan null mutant follicle cells have normal apical-basal polarity. However, mutants in Dystroglycan or in its extracellular matrix ligand Perlecan lose polarity under energetic stress. Starved Dystroglycan or Perlecan null cells activate AMPK normally, but do not activate and do not properly localize Myosin II. Thus, Perlecan signaling through Dystroglycan may determine where Myosin II can be activated, thereby providing the basal polarity cue for the low-energy epithelial polarity pathway (Mirouse, 2009).
Finally, our current work suggests that AMPK is indeed required in vivo for Myosin II phosphorylation under energetic stress but that this phosphorylation could be not direct but rather may involve the Rho kinase.

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RAB8-MEDIATED RHO GTPase ACTIVITY AND FOCAL ADHESION TURNOVER REGULATES DIRECTIONAL CELL MIGRATION

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Rab8 is a small Ras-related GTPase that regulates polarized membrane transport to the plasma membrane and promotes cell protrusion. Our previous studies demonstrated the involvement of Rab8 in MT1-MMP-dependent tumour cell invasion (Bravo-Cordero JJ et al., 2007). Since migration is key during invasion, we addressed the role of Rab8 GTPase in the migratory process. Rab8 shRNA silenced cells showed compromised directional persistency of cell motility, chemotaxis towards EGF gradients, and wound closure. These cells showed changes in cell polarity and displayed a dramatic increase in actin stress fibres. Conversely, Rab8 constitutively active mutant induced cortical actin polymerization and greatly diminished actin stress fibres. We uncovered the molecular mechanism responsible for Rab8 dependent actin cytoskeletal rearrangements which relied on Rac activation and Rho inhibition. As revealed by pull-down assays, Rab8 activation increased Rac GTP-loading, while depletion of Rab8 activated Rho. Moreover, Rab8 was associated with microtubules and FA being also involved in focal adhesion turnover, promoting disassembly of these structures independently of Rho GTPases. Altogether, these data reveal a novel role of Rab8 in cell motility by regulating the establishment of cell polarity, turnover of focal adhesions and actin cytoskeleton rearrangements thereby determining directionality of cell migration.

Motor neuron degeneration in wobbler mice and embryonic lethality in Vps54 knock out indicates the importance of functional retrograde vesicle transport

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The wobbler mouse is an intensively investigated animal-model for human motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Besides motor neuron degeneration the wobbler mutation causes a spermiogenesis defect similar to human globozoospermia. Recently, we identified a point mutation in the vesicle traffic factor Vps54, which is responsible for the motor neuron degeneration in wobbler mice (1). The wobbler point mutation is a hypomorphic allele of Vps54, because Vps54 knock out causes embryonic lethality around day 11 of embryonic development. Homozygous Vps54 embryos show retarded development, nearly absent dorsal root ganglia and an abnormal membrane blebbing seen at the luminal surface of the ependyma.
Since Vps54 is an ubiquitously expressed vesicle traffic factor involved in the tethering of endosome-derived vesicles to the Trans Golgi membrane we investigated the retrograde vesicle transport in embryonic fibroblasts isolated from wildtype, wobbler and Vps54 -/- embryos. The uptake and retrograde transport of choleratoxin as well as the distribution of manose-6-phosphate receptors was fond to be altered in Vps54 mutant cells, indicating a defect in retrograde vesicle traffic.
The impairment of the retrograde vesicle traffic might also be responsible for the abnormally enlarged endosomal compartments we found in degenerating motor neurons of wobbler mice but not in SOD1 G93A transgenic mice, an animal model for familial ALS. Interestingly, we could identify wobbler-like enlarged endosomal structures in the degenerating spinal motor neurons of a subset of sporadic ALS cases. Thus, we think that retrograde vesicle traffic is critical for motor neuron disease in wobbler mice and, at least for a subset of human ALS cases.
(1) Schmitt-John et al. (2005) Mutation of Vps54 causes motor neuron disease and spermiogenesis defect in the wobbler mouse Nat. Genet. 37: 1213-1215.

Sec6p anchors the assembled exocyst complex at sites of secretion

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The exocyst is an essential protein complex required for targeting and fusion of secretory vesicles to sites of exocytosis at the plasma membrane. To study the function of the exocyst complex at the molecular level, we performed a structure-based mutational analysis of the Saccharomyces cerevisiae exocyst subunit Sec6p. Two “patches” of highly conserved residues are present on the surface of Sec6p; mutation of either patch does not compromise the stability of the protein. Nevertheless, replacement of the wild-type Sec6p with the patch mutants results in severe temperature-sensitive growth and secretion defects. At non-permissive conditions, although trafficking of secretory vesicles to the plasma membrane is unimpaired, none of the exocyst subunits are polarized at sites of secretion. This is consistent with data from other exocyst temperature sensitive mutants, which disrupt the integrity of the complex. Surprisingly, however, these patch mutations result in mislocalized exocyst complexes that remain intact. Our results indicate that assembly and polarization of the exocyst are functionally separable events, and that the conserved patches on the surface of Sec6p are required to anchor exocyst complexes to sites of secretion.

Unconventional Trafficking and Subsequent Behavior of the Polar Landmarks Bud8p and Bud9p in Yeast

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In S. cerevisiae, the homologous integral-membrane proteins Bud8p and Bud9p, which form landmarks for bud emergence, localize specifically to the birth-scar-distal (Bud8p) and -proximal (Bud9p) poles, respectively. We have been exploring the mechanisms of this asymmetric organization. Both proteins have large extracellular regions (>450 a.a.) at their N-termini without signal-peptide sequences. Given this uncommon structure, it has been thought that the trafficking of these proteins might be unconventional. We explore two aspects of this here. First, both proteins localize to their respective poles with remarkable stability and can act as budding cues even in cells arrested in G1 for >20 days. This suggests that they may be incorporated into atypical, extremely stable membrane domains. Neither protein colocalizes with either Pma1p or Can1p (two known components of patch-like lipid rafts in the plasma membrane), although previous evidence suggests that both Bud8p and Bud9p are associated with lipid rafts of some type. Second, glycosylation of both proteins occurs only ~30 min after pulse expression, suggesting that there is a substantial delay between protein synthesis and translocation into the ER. During this lag phase, Bud8p and Bud9p already begin to localize to the cell poles, in apparent contrast to the conventional pathway, in which proteins are sorted to appropriate destinations at the TGN.

The atypical secretion proteins Bud7p and Chs5p are involved in the trafficking of both Bud8p and Bud9p. Bud7p is one of the ChAPs (Chs5p-Arf1p-binding proteins), which are localized primarily to the TGN and appear to be involved in exocytosis from the TGN to the cell surface. However, depletion of Arf1p (the small GTPase required for CCV and COPI coat assembly) does not affect the budding-pattern phenotype. Moreover, surprisingly, when we asked where in the Bud8p/Bud9p trafficking pathway Bud7p is required, we found that in the absence of either Bud7p or Chs5p, Bud8p and Bud9p appear to sort directly to the vacuole from the cytoplasm and degrade. It is unclear how proteins that seem to localize in the late trafficking pathway can affect an early step in protein trafficking. We did also find that Bud7p is required for a later stage in trafficking (somewhere during ER exit, passage through the Golgi, and/or delivery to the plasma membrane). Thus, it appears that Bud7p acts to keep Bud8p and Bud9p on the correct, but apparently unusual, track throughout their trafficking to the cell surface.

Caveolin-1 recruitment mediates Rac1 degradation

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Directional cell migration is critically dependent on the spatio-temporal control of intracellular signalling events. These control cytoskeletal dynamics which provides both driving force, mediated by actin polymerization at the front and contraction at the rear, as well as directionality and persistence, mediated by polarized transport of vesicles and proteins along microtubules. The actin and microtubule cytoskeleton are controlled by, and provide feedback input to Rho-like small GTPases. The best studied members of the RhoGTPase family are CDC42 and Rac1, which promote actin polymerization and cell protrusion, and RhoA, which stimulates myosin-based contractility. Like most other small GTPases, the Rho family members act as molecular switches, cycling between an active, GTP-bound state and inactive, GDP-bound state. Although GTP-loading is critical for GTPase signalling, constitutive activation of Rho GTPases blocks, rather than stimulates, responses such as polarization or migration. Thus, it appears that properly controlled, localized inactivation of GTPases is important for efficient cellular responses. The GTPase-activating proteins (GAP) proteins play an important role in this as they enhance the low intrinsic GTP-hydrolysing activity of the small GTPases. However, accumulating evidence suggests that, in addition to GAP-mediated GTPase inactivation, additional mechanisms exist to switch off GTPase signaling.

Recently, we identified the scaffolding protein Caveolin-1 (Cav1) to selectively bind the C-terminus of Rac-1. Cav1 has been identified as a marker of caveolae, small membrane invaginations that regulate membrane organization and cell signalling. Cav1 has been implicated in many functions, including regulation of cell migration. In this study we show that activation of endogenous Rac1 induces the recruitment of endogenous Cav1 to peripheral sites of adhesion, where Rac1 and Cav1 colocalize. Moreover, reduction of endogenous Cav1 levels by siRNA transfection induces a significant increase in cell spreading and impairs cell migration. Intriguingly, in Cav1-depleted cells activation of Rac1 is enhanced, parallel to an increase in total Rac1 levels. Further analysis showed a significant increase in endogenous mono-ubiquitinated Rac1 in Cav1-knockdown cells. This suggests that Cav1 plays a role in degradation of mono-ubiquitinated Rac1, explaining the accumulation of total cellular levels of Rac1 in the absence of Cav1. In conclusion, these data show that Cav1 is a negative regulator of Rac1 expression levels and signalling output. Moreover, these findings reveals an additional level of regulation of Rac1 signaling, based on ubiquitination and Cav1-mediated degradation of activated Rac1.

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Membrane trafficking in cytokinesis: a role for the exocyst

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Membrane trafficking and the establisment of polarised PM domains are essential for cytokinesis. The exocyst complex regulates the tethering and fusion of vesicules at polarised PM domains leading to abscission. Although all the exocyst components localise to the midbody at the final stages of cytokinesis we have the first evidence that the different exocyst components reach the abcission site via distinct trafficking pathways.

The RhoGAP SRGP-1 regulates cell killing and clearance through CED-10/Rac1 in C. elegans

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The proper removal of apoptotic cells is critical during embryonic development and in tissue homeostasis to prevent inflammation and autoimmunity. Phagocytes recognize apoptotic cells and start to re-orchestrate their cytoskeleton and membranes towards the corpse to actively support apoptosis and furthermore ensuring its clearance. However, the molecular details of the “engulfment machinery” are not fully understood. The powerful genetics of the small nematode C. elegans has been used to identify genes involved in the clearance of apoptotic cells during development and in the adult germ line. Ten genes were isolated which act in two partially redundant pathways. One group is composed of five genes: the small GTPase MIG-2/RhoG and its nucleotide exchange factor UNC-73/TRIO, as well as the adaptor protein CED-2/CrkII and the bipartite RacGEF complex CED-5/Dock180 and CED-12/ELMO. In the second pathway, CED-1/CD91/LRP/SREC/EATER functions as a transmembrane receptor which might recognize the apoptotic cell. The CED-7/ABCA1 transporter is likely important for membrane dynamics, and plays an additional critical role in dying cells. CED-6 and its human homologue GULP encode a signaling adaptor molecule which physically interact with CED-1. DYN-1/Dynamin2 regulates the vesicular traffic downstream of CED-6. Both pathways converge at the level of CED-10/Rac. However, the signaling pathways between CED-6, DYN-1 and CED-10, as well as events downstream of CED-10 have yet to be identified. Furthermore, a second yet unknown signaling pathway that acts in parallel to CED-10 remains to be identified.
Using the combined approach of the vital dye Acridine Orange (AO, which visualizes engulfment activity) and RNAi, we identified srgp-1, a RhoGEF which re-allows AO staining of ced-6 or ced-5 germ cell corpses and suppress persistent corpse numbers during development. srgp-1 acts in the engulfing cell, which is supported by SRGP-1::GFP localization. The loss of srgp-1 results in increased engulfment kinetics and srgp-1 over-expression enhances engulfment defects. Epistasis places srgp-1 downstream or in parallel to ced-10. We will show evidence that SRGP-1 binds and regulates CED-10 GTPase activity. Taken together, the loss of srgp-1 resembles an increased engulfment signaling background - which indeed can promote apoptosis of cells that fail to fully undergo programmed cell death. This "promotion" of engulfment might be a prerequisite for therapeutics designed to induce the clearance of “unwanted” cells.

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Characterization of the Myo4p-cargo complex from yeast - implications for mRNA and ER transport

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Motor protein-dependent transport and localization of mRNA is essential for cellular asymmetry, cell differentiation, and embryogenesis. Attempts to purify the components of localizing mRNPs usually result in the identification of dozens of proteins and hundreds of RNAs. The large number of involved factors and the assumed heterogeneous nature of purified mRNPs makes a molecular characterization of mRNP assembly a challenging task. A major exception is the localization of ASH1 mRNA and about 30 additional transcripts during mitosis of S. cerevisiae. Here, all mRNP-core factors have been identified. In addition to mRNA localization, this transport complex mediates the inheritance of endoplasmic reticulum (ER). Thus, yeast is a comparably simple model system to study the combined transport of mRNA and membrane organelles.

Motile activity of this transport complex is mediated by the type V myosin Myo4p. Type V myosins have been shown to bind their cargo via their C-terminal globular tail. Consistently, Myo4p is known to bind the adapter protein She3p through a larger region that includes the globular tail. She3p then interacts with the RNA-binding protein She2p to form the core mRNP. Although ER inheritance requires Myo4p and She3p, the RNA-binding protein She2p is dispensable for this process. Thus, the assembly of the Myo4p-She3p co-complex is the central interaction for the transport of mRNA as well as ER.

Here we report a multi-disciplinary study characterizing the cargo binding of Myo4p. To our surprise, we found that the binding of the She3p adapter to Myo4p only involves regions outside the globular tail. This result raises the question what the function of the Myo4p globular tail might be instead of cargo binding. Subsequent in vivo studies showed that the myosin globular tail is also dispensable for ER inheritance. In contrast, mRNA localization is impaired in cells expressing a Myo4p fragment that lacks the globular tail. By using in vitro binding assays with the Myo4p globular tail we observed a stable interaction with membrane vesicles. Using high-resolution structural analyses we identified a potential membrane-binding region in the globular tail. The relevance of this region was confirmed by mutations that abolish membrane binding. In summary, our results show that the globular tail is not involved in cargo binding. Instead, our results suggest that the Myo4p globular tail is required for mRNP localization at the stage of membrane binding and mRNP anchoring at the bud tip.

Different GPI-attachment signals modulate GPI-anchored protein oligomerization and apical sorting

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Glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) are directly targeted to the apical domain of plasma membrane of the majority of epithelial polarized cells and partition preferentially in dynamic membrane domains enriched in sphingolipid and cholesterol called rafts or DRM (detergent resistant membrane) because of their resistance to detergent extraction. We have recently demonstrated that GPI-APs travel to the apical membrane of polarized epithelial cells in high molecular weight complexes and that impairment of their oligomerization leads to their basolateral missorting.
However, the mechanism responsible for apical GPI-AP oligomerization is still unknown, and both the ectodomain and GPI-anchor and/or lipid environment could be involved. In order to understand the role of the GPI-anchor in the oligomerization and consequently in the apical sorting of GPI-APs we have fused at the C-terminus of the green fluorescent protein (GFP), two different GPI attachment signals respectively derived from an apically sorted GPI-AP, the folate receptor (FR) and from a basolaterally sorted one, the prion protein (PrP). We have studied the behaviour of the resulting fusion proteins, GFP-FR and GFP-PrP, stably transfected in MDCK cells by analysing three parameters: DRM-association, oligomerization and apical sorting. Strikingly we found that different GPI-attachment signals modulate the ability of the resulting GFP-fusion proteins to oligomerize and to be apically sorted. This is likely due to the attachment of a structurally different GPI anchor. Consistently with these data, using a FRAP approach we found that, at the level of the Golgi apparatus, GFP-PrP displays a higher apparent coefficient diffusion (D) compared to GFP-FR in agreement with the fact that GFP-PrP is monomeric, while GFP-FR forms high molecular weight complexes. Interestingly we found that addition of cholesterol is able to “convert” a non-oligomerizing basolateral protein into an oligomerizing apical one. Indeed, upon cholesterol addition the D of GFP-PrP decreased significantly, and the protein was then able to oligomerize and was re-directed to the apical cell surface, thus suggesting that a specific lipid environment is required for oligomerization and consequently for apical sorting of GPI-APs.
Altogether our findings suggest that in order to be apically sorted a GPI-AP should have two properties: 1) to partition into a favourable environment that allows oligomerization to occur; 2) to have an intrinsic capability to form high molecular weight complexes. We propose that these two properties derive from the integrated action of the GPI anchor and the ectodomain of the protein.

In vivo phosphorylation of Epsilon adaptin occurs at different sites

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AP-4 is a cytosolic protein, member of the “adaptor protein” family, like AP-1, AP-2 and AP-3. It is an heterotetrameric complex composed by 4 subunits with different characteristics: Epsilon 127 KDa, beta-4 83 KDa, mu-4 50 KDa and sigma-4 30 KDa.
The Adaptor complex AP-4 is predominantly localized in the Trans Golgi Network (TGN) region and it seems to play a role in the protein transport from TGN to lysosomes and endosomes. It also seems to be involved in the basolateral sorting in MDCK cells and in protein trafficking in neuronal cells.
Every different subunit composing the entire complex, seems to have a particular function: beta-4 , compared with the homologous of AP-1, AP-2 and AP-3, is truncated and lacks the clathrin binding domain. No cytosolic binding partners have been discovered yet and beta-4 could have lost its recruitment function; mu-4, instead, has shown the ability to recognize and bind chanonical sorting signals such as tyrosin and di-leucine based motives present on different proteins like LDRL, MPR-46, Lamp-1.
We speculated that something new, about AP-4 function could emerge by studying the epsilon adaptin appendage domain. In fact it has been demonstrated that the alfa ear domain of AP-2 interacts with numerous molecules that regulate clathrin coat assembly or vesicle formation and many coat proteins are phosphorylated reversibly in vivo like the alfa-appendage domain, in which more than one functional phosphorylation site have been identified.. Furthermore the complex endocytic process is tightly regulated by phosphorylation / dephosphorylation cycles. In the present study we show that the cellular distribution of the heterotetrameric complex AP-4 is regulated by phosphorylation / dephosphorylation events, indeed this cycle is controlled by the PP2A like protein phosphatases family and by staurosporine sensitive kinases. We could also show that phosphorylation at different serine threonine sites of the hinge / ear region is critical for molecular charge modifications and this process needs more than one actively phosphorylated site. A critical region, encompassing aa 839 to aa 871 is involved in the regulation of AP-4 / membranes interactions and finally we characterized three different phosphorylation sites in this region that act cooperatively on the epsilon ear molecular charge modification.
These are the first data on AP-4 phosphorylation, indicating a tightly modulated mechanism and also suggesting a dynamic function for this heterotetrameric adaptor protein complex.

Polarization of the Cdc42 GTPase mediated by its effectors and its upstream GTPase

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Establishment of cell polarity requires the polar distribution of the Rho-type GTPase Cdc42, which is essential for cytoskeletal polarization. Cells of the budding yeast Saccharomyces cerevisiae undergo oriented cell division by choosing a specific bud site on the cell cortex. The Ras-type GTPase Rsr1 (also known as Bud1) and its regulators determine the axis of polarized cell growth by linking a spatial landmark to the Cdc42 module. Cdc42 polarization can also occur in the apparent absence of a spatial cue, through a process called symmetry breaking. Here we report that Rsr1 and two related Cdc42 effectors, Gic1 and Gic2, share a role in mediating Cdc42 polarization. Cells lacking Rsr1, Gic1, and Gic2 failed to establish polarization of active Cdc42. Rsr1 interacted with Cdc42 and with itself, and these interactions required an intact polybasic domain of Rsr1. These GTPase interactions as well as the cycling of Rsr1 between the GTP- and GDP-bound states were necessary for Cdc42 polarization. A cdc42 mutant protein that poorly interacted with Gic1/Gic2 failed to cluster at a single site, despite its localization to the plasma membrane. These results thus indicate that Cdc42 polarization is established by Rsr1 and Gic1/Gic2. Gic1 and Gic2, which interact with phospholipid, may contribute to the stabilization of active Cdc42 on the plasma membrane, leading to clustering of active Cdc42 at a single site. Given that Gic1 and Gic2 are downstream targets of Cdc42, our findings also reveal a positive feedback mechanism for Cdc42 polarization.

Structural and functional polarity of a single celled organism, Paramecium tetraurelia

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Polarity of single celled organisms, such as the ciliated protozoan, P. tetraurelia, may be determined epigenetically and, thus, difficult to approach by the usual methodologies. In P. tetraurelia the steadily ongoing turnover/biogenesis of the contractile vacuole complex (CVC, two per cell) and particulalrly the unilateral (polar) de novo formation of one CVC preceding each cell division is such a phenomenon that has not been analyzed previously. Now CVC biogenesis is amenable to structural and functional analysis by identification and localization of its proteins via antibody staining and expression as GFP fusion proteins, in parallel to post-translational homology-dependent gene silencing. This has been performed with NSF (N-ethylmaleimide sensitive factor), SNAREs (SNAP receptors; SNAP = soluble NSF attachment protein) of which synaptobrevin 2 and syntaxin 2 are restricted to the CVC, subunits (SUs) of the H+-ATPase (some SUs also being CVC-specific) and an inositol 1,4,5-trisphosphate receptor (IP3R), also localized specifically to the CVC. We find an unexpected extent of membrane turnover in the CVC with a mutual dependence of the localization of some – though not all - of these components to the CVC and their turnover. These observations support a novel concept, i.e., CVC biogenesis by “silent”, organelle-directed and organelle-specific vesicle trafficking, while in ultrastructural studies no such vesicle trafficking has ever been seen. - Supported by Deutsche Forschungsgemeinschaft.

Rip11/FIP5 and Rab11 Binding to Sorting Nexin 18 Regulates Apical Endocytic Protein Transport in Polarized Epithelial cells

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Epithelial cells are structurally and functionally polarized to transport specific molecules selectively and uni-directionaly while maintaining trans-epithelial barrier. This selective transport is achieved by a junctional complex that partitions the plasma membrane into two distinct domains: apical and basolateral, with both of these plasma membrane compartments having distinct lipid and protein compositions. Apical transport is fundamental to the function of all epithelial cells and malfunctions in a number of diseases. Rab11 GTPases are members of the small monomeric GTPase super-family that have been implicated in regulating apical endocytic transport. In the last few years several Rab11-binding proteins have been identified, including the Rab11 family interacting proteins, also known as FIPs. In this study we show that Rip11/FIP5 is highly enriched at the apical pole of polarized MDCK cells as well as in the rat kidney tubules. Furthermore, we use MDCK cell line, stably-expressing tet-inducible Rip11/FIP5 shRNA, to demonstrate that Rip11/FIP5 plays a key role in protein transport to the apical plasma membrane in polarized 2D and 3D cultures. Furthermore, we used electron microscopy analysis of Rip11/FIP5 dominant negative mutant expressing cells to show that Rip11/FIP5 is required for the protein exit from tubulo-vesicular recycling endosomes.
Work from several laboratories, including our own, have shown that FIPs act as scaffolding factors allowing the assembly of specific sorting/transport complexes required for the endocytic protein traffic. Thus, used proteomics to identify Sorting Nexin 18 (SNX18) as a Rip11/FIP5-binding protein. We have mapped Rip11/FIP5 and SNX18 binding motifs and shown that Rip11/FIP5 binding induces SNX18-dependent endosome tubulation in vitro and in vivo. Finally, we have used tet-inducible SNX18 shRNA MDCK cell line to demonstrate that SNX18 in also required in apical protein transport in 2D and 3D cultures. Based on these data, we propose that the binding of Rab11 and Rip11/FIP5 protein complex to SNX18 activates SNX18-dependent budding/sorting of apical endocytic proteins carriers in polarized epithelial cells.

Role of the v-SNARE Cellubrevin/VAMP3 in epithelial polarity

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Cell polarity requires the establishment and maintenance of plasma membrane subdomains. Migrating cells exhibits horizontal polarity with a leading edge at the front of the cell and a retracting rear edge. Polarized epithelial cells have vertical polarity with apical and basolateral membranes of different protein and lipid compositions. Phosphoinositide 3-kinase (PI(3)K) and its lipid product phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P(3)) regulate cell migration and polarity. PI(3,4,5)P3 stably localizes at the basolateral plasma membrane and is excluded from the apical membrane. It regulates the formation of the basolateral plasma membrane in epithelial cells. The link between membrane trafficking and PI(3)K is still largely unknown.
We have previously shown that membrane trafficking driven by the early endosomal v-SNARE Cellubrevin (Cb, also called VAMP3) is involved in cell migration via the regulation of ß1-integrin-dependent cell adhesion. Cb-deficient cells (expressing tetanus neurotoxin light chain, which selectively cleaves cellubrevin) migrate and spread slower, but adhere faster. ß1-integrin endocytosis is reduced in Cb-deficient cells during cell migration whereas endocytosed ß1-integrin colocalise with Cb in normal cells. Furthermore, a subset of basolateral proteins, corresponding to clathrin adaptor protein AP-1B-dependent cargos such as transferrin receptor, is delocalised to the apical pole in polarised Cb-deficient cells.
Here we report the role of Cb in the establishment of a polarized epithelial monolayer. We show polarity defects in Cb-deficient MDCK epithelial cells grown on filters with striking irregular monolayer and increased cell height. Cb-deficient cells grow to higher cell density but their proliferation rate is unchanged. Apical proteins and the tight junction protein ZO-1 are still well localised, and acquisition of trans-epithelial resistance is unchanged in these cells suggesting that tight junctions are functional and develop normally. Further, we show over-activation of PI(3)K and remodelling of actin in Cb-deficient cells. The observed phenotype of irregular monolayer and actin remodelling is reverted by treatment with PI(3)K inhibitor LY294002. These results indicate that Cb regulates epithelial cell polarity likely via the regulation of a polarized PI(3)K-dependent activity.

Polarization of dendritic cells at the immunological synapse facilitate local delivery of T cell priming cytokines

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The adaptive immune response depends on the ability of antigen presenting cells (APC) to transmit the information about the invading pathogen to effector T cells. Understanding the molecular mechanisms that control informational transfer between the two cells at the
immunological synapse (IS) is a major challenge for the cell biology of the immune system. We have investigated polarization of the microtubule cytoskeleton (MT) and polarity proteins in DCs forming synapses with naïve T cells. Our results show that in antigen specific synapses DCs orient the MT organizing center (MTOC) toward the interacting T cell. IL-12, a pivotal cytokine for T cell priming, is concentrated around the MTOC in TLR activated DCs and is translocated to the DC-T contact site.
Our data suggest that IL-12 is secreted in a polarized fashion toward the interacting T cell. Synaptically delivered IL-12 induces STAT4 signalling in T cells engaged in antigen specific synapses. These results reveal a novel mechanism by which dendritic cells control initiation of adaptive immune responses.

Hsp90 co-localizes with rab-GDI-1 and regulates agonist-induced amylase release in AR42J cells

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Rab proteins are small GTPases required for vesicle trafficking through the secretory and endocytic pathways. Rab GDP-dissociation inhibitor (rab-GDI) regulates Rab protein function and localization by maintaining Rab proteins in the GDP-bound conformation. Two isoforms of rab-GDI are present in most mammalian cells: GDI-1 and GDI-2. It has recently been demonstrated that a Heat shock protein 90 (Hsp90) chaperone complex regulates the interactions between Rab proteins and Rab-GDI-1. The AR42J cell line is derived from rat pancreatic exocrine tumor cells and develops an acinar-like phenotype when treated with dexamethasone (Dex). The aim of the present study was to examine the expression of rab-GDI isoforms and Hsp90 in AR42J cells in the presence and absence of Dex. Rab-GDI:Hsp90 interactions were also examined. Both rab-GDI isoforms were detected in AR42J cells by immunoblotting. In Dex-treated cells, quantitative immunoblotting revealed that rab-GDI-1 expression increased by 28%, although this change was not statistically significant. Rab-GDI-2 levels were unaltered by Dex treatment. Approximately 21% rab-GDI-1 was membrane associated, whereas rab-GDI-2 was exclusively cytosolic. Dex treatment did not affect the subcellular distribution of rab-GDI isoforms. Hsp90 was present in the cytosolic and membrane fractions of AR42J cells. In contrast to what others have observed, Hsp90 did not co-immunoprecipitate with cytosolic rab-GDI-1. However, density gradient centrifugation of AR42J cell membranes suggests that Hsp90 and rab-GDI-1 co-localize to the same membrane compartment. The Hsp90 inhibitor, geldanamycin, inhibited CCK-8-induced amylase release from these cells by 58%. Our results indicate that as AR42J cells differentiate into acinar-like cells, rab-GDI isoform expression and localization is not significantly altered. Moreover, our findings suggest that Hsp90 regulates agonist-induced secretion in exocrine cells and may interact with rab-GDI-1 on acinar cell membranes.

Basolateral sorting of Syntaxin-4 in polarized cells: effect of m1B

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Generation of polarity requires mechanisms to sort the plasma membrane proteins into apical and basolateral domains. In polarized epithelial cells, Syntaxin-4 localizes exclusively to the basolateral plasma membrane and plays an important role in membrane fusion of basolateral trafficking pathways. Though the localization of this protein is known from a long time, the sorting signal and sorting mechanism has not been completely depicted yet. Analysis of kinetics of Syntaxin-4 delivery in Madin-Darby canine kidney (MDCK) cells demonstrated that newly synthesized protein is delivered to basolateral membrane only and not re-directed to both membranes. We used deletion mutation approach to identify sorting signal for the basolateral delivery of Syntaxin-4 in MDCK cells. Deletion of first 29 N-terminal amino acids resulted in non-polarized location of Syntaxin-4 and deletion of first 10 or 24 amino acids did not result in the mislocalization of Syntaxin-4. This indicates that the region between residues 24-29 N-terminal of Syntaxin-4 is critical for its basolateral localization. Besides, we demonstrate by single and multiple tyrosines mutated to an alanine that probable tyrosine signal is not involved in basolateral sorting of Syntaxin-4. Expression of mislocalized mutant 29 N-terminal Syntaxin 4 in MDCK results in the inability to form organized cysts but leads not disturbance of tight junction formation.
Epithelial cell specific adaptor complex AP1B has been reported to involve in basolateral trafficking of many proteins. We took advantage of LLC-PK1 cells, which naturally lack component m1B of AP1B complex, to study if AP1B is required for the basolateral sorting of Syntaxin-4. These cells were stably transfected to express either m1A or µ1B subunit of AP1B. Syntaxin-4 is localized in a non-polarized manner to both apical and basolateral membranes in the cells expressing m1A (lacking µ1B), but localizes to basolateral membrane in cells that express µ1B. It demonstrates that µ1B is involved in basolateral sorting of Syntaxin-4. Furthermore, we confirmed the interaction of µ1B and Syntaxin-4 in vivo, using MDCK stable transfected with HA-Syntaxin-4 infected with myc tagged µ1B viruses.
These results indicate that the polarity of Syntaxin-4 is essential for epithelial cell polarization and AP1B mediates the basolateral sorting of this protein.

Membrane invaginations reveal sites of force generation in C.elegans embryos

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During cell division in C.elegans embryos, the mitotic spindles are positioned by forces acting on microtubules at the cell cortex. In order to directly visualize sites of force generation, we weakened the cortex by RNAi against of components of the acto-myosin machinery. This led to the formation of membrane invaginations that appeared to be pulled from the cortex towards the spindle poles. These invaginations depend on microtubules and molecules known to be required for microtubule dependent force generation on the spindle. The asymmetric distribution of invaginations mirrors the anterior-posterior asymmetry of force generation. We therefore argue that invaginations correspond to the sites of force generation and that these force generators are anchored in the cell membrane. We propose that the actomyosin cytoskeleton stabilizes the membrane against these pulling forces, allowing the force generated by pulling microtubules to be transmitted efficiently to the spindle. Consistent with this hypothesis, weakening the cortex disturbs spindle positioning.

A conserved family of endocytic adaptors that coordinate cargo selection and vesicle formation

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Internalization of transmembrane cargos from the plasma membrane requires a diverse array of specialized endocytic adaptors, yet only a few adaptors are known. The yeast protein Syp1 has been implicated in endocytosis through its subcellular localization and binding partners. We solved structures of two previously unidentified domains in Syp1; an N-terminal EFC/F-BAR domain with membrane tubulating activity, and a C-terminal cargo-binding mu Homology Domain (μHD) similar to mu-adaptin subunits of AP complexes. Three mammalian homologues of Syp1 were also found: FCHO1, FCHO2 and SGIP1alpha.
Our recent studies with Syp1 and its homolog FCHO1 are testing the hypothesis that Syp1 defines a new family of endocytic adaptors. Consistent with our hypothesis, the EFC domain of Syp1 binds to liposomes, and mediates both tubulation and vesiculation. The μHDs of this family show conserved interactions with the endocytic adaptor/scaffold Ede1/eps15, which contributes to in vivo localization of Syp1. In vitro and in vivo data show that the Syp1 μHD interacts with a transmembrane protein cargo previously implicated in polarized Rho1 signaling. We propose that this novel family of adaptors directly link cargo selection with vesicle scission, thereby regulating receptors that stimulate cell polarization pathways.

In vivo phosphorylation of Epsilon adaptin occurs at different sites

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Text L. Paolini

AP-4 is a cytosolic protein, member of the “adaptor protein” family, like AP-1, AP-2 and AP-3. It is an heterotetrameric complex composed by 4 subunits with different characteristics: Epsilon 127 KDa, beta-4 83 KDa, mu-4 50 KDa and sigma-4 30 KDa.
The Adaptor complex AP-4 is predominantly localized in the Trans Golgi Network (TGN) region and it seems to play a role in the protein transport from TGN to lysosomes and endosomes. It also seems to be involved in the basolateral sorting in MDCK cells and in protein trafficking in neuronal cells.
Every different subunit composing the entire complex, seems to have a particular function: beta-4 , compared with the homologous of AP-1, AP-2 and AP-3, is truncated and lacks the clathrin binding domain. No cytosolic binding partners have been discovered yet and beta-4 could have lost its recruitment function; mu-4, instead, has shown the ability to recognize and bind chanonical sorting signals such as tyrosin and di-leucine based motives present on different proteins like LDRL, MPR-46, Lamp-1.
We speculated that something new, about AP-4 function could emerge by studying the epsilon adaptin appendage domain. In fact it has been demonstrated that the alfa ear domain of AP-2 interacts with numerous molecules that regulate clathrin coat assembly or vesicle formation and many coat proteins are phosphorylated reversibly in vivo like the alfa-appendage domain, in which more than one functional phosphorylation site have been identified.. Furthermore the complex endocytic process is tightly regulated by phosphorylation / dephosphorylation cycles. In the present study we show that the cellular distribution of the heterotetrameric complex AP-4 is regulated by phosphorylation / dephosphorylation events, indeed this cycle is controlled by the PP2A like protein phosphatases family and by staurosporine sensitive kinases. We could also show that phosphorylation at different serine threonine sites of the hinge / ear region is critical for molecular charge modifications and this process needs more than one actively phosphorylated site. A critical region, encompassing aa 839 to aa 871 is involved in the regulation of AP-4 / membranes interactions and finally we characterized three different phosphorylation sites in this region that act cooperatively on the epsilon ear molecular charge modification.
These are the first data on AP-4 phosphorylation, indicating a tightly modulated mechanism and also suggesting a dynamic function for this heterotetrameric adaptor protein complex.

Down-regulation of Death-Associated Protein Kinase-2 is required for beta-catenin-induced anoikis resistance of malignant epithelial cells

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Resistance of solid tumor cells to anoikis, apoptosis induced by cell detachment from the extracellular matrix, is thought to be critical for the ability of these cells to grow anchorage independently within thee-dimensional tumor masses and from metastases. beta-catenin, a major oncoprotein, can inhibit anoikis of cancer cells via unknown mechanisms. In an effort to identify these mechanisms we found that beta-catenin blocks anoikis of malignant kidney and intestinal epithelial cells and promotes their anchorage-independent growth by down-regulating death-associated protein kinase-2 (DAPk-2), a pro-apoptotic protein whose cellular functions have so far remained unexplored. We found that beta-catenin-induced down-regulation of DAPk-2 requires the presence of the transcription factor Tcf-4, a known mediator of beta-catenin signaling. We also observed that DAPk-2 contributes to the execution of anoikis of the non-malignant epithelial cells. Thus, beta-catenin-induced down-regulation of DAPk-2 represents a novel signaling mechanism by which beta-catenin promotes the survival of malignant epithelial cells following their detachment from the ECM and enables these cells to grow in an anchorage-independent manner.

Kinetic characterization of clathrin cage disassembly by the molecular chaperone Hsc70 and its partner auxilin

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Clathrin mediated endocytosis is a fundamental function of eukaryotic cells. It is important for nutrient uptake, protein trafficking, cell signalling and development. In a coordinated process involving many different proteins, a clathrin-coated invagination is formed on the cytoplasmic face of the plasma membrane, which eventually buds off to form a clathrin coated vesicle carrying protein cargo. Before the vesicle can fuse with its intracellular target membrane the cage has to be removed. This uncoating of clathrin coated vesicles is achieved by the chaperone protein Hsc70 and its partner auxilin, in an ATP-dependent process.
Using purified protein components and biophysical methods our aim is to examine the mechanism of clathrin cage disassembly by Hsc70 and auxilin, to determine the kinetics of the disassembly, and dissect the function of the individual components.
Clathrin is composed of a heavy chain and a light chain. Three clathrin molecules come together to form a ‘three-legged’ triskelion. In vitro, these triskelions can form cages in the absence of lipid. Using a novel assay based on light scattering, we have shown that disassembly occurs quickly, with a maximal rate of 0.065 s-1 (t1/2 ≈ 10 s). Whilst excess Hsc70 is required for the maximal rate of disassembly, auxilin is only required at a ratio of one per triskelion (three clathrin molecules). ATP hydrolysis is required not only for the cage disassembly but for the clathrin:auxilin:Hsc70 complex to form, and ATP is hydrolysed during the disassembly process at a rate of 1 mol per mol of clathrin disassembled. These results, together with previously published data, suggest that a single auxilin bound to three clathrin molecules (or one triskelion) somehow recruits three Hsc70 molecules which each hydrolyse one ATP during the disassembly process. Yet isothermal titration calorimetry shows that Hsc70 and auxilin bind at a ratio of 1:1 in the absence of clathrin. Future work aims to examine the interactions using fluorescence anisotropy, to try to understand the mechanism underlying auxilin’s recruitment of Hsc70, and how Hsc70 removes a clathrin triskelion from a cage.

Poster 86

A minimalistic EGFR ligand trafficking and processing machinery in the flour beetle

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Intra-cellular trafficking plays a critical role in the Drosophila EGF receptor(EGFR)pathway, in regulation of the ligand processing machinery by intra-membrane proteolysis. Recent work in the lab has uncovered a new mode of ligand processing: In the eye and germline, localization of the protease, Rhomboid, which generates the active EGFR ligands, encompasses both the ER and a late secretory compartment. The protease exerts opposite effects in the two compartments. In the ER, premature cleavage of the chaperone Star, attenuates the amount of ligand that can be productively trafficked to the late compartment. Conversely, in the late compartment, the protease generates the active cleaved ligand that is secreted.

We examined the ligand processing cassette in the flour beetle Tribolium, where whole genome sequencing has shown only a single Rhomboid, EGFR ligand, and chaperone. Tribolium Rhomboid behaves like the Drosophila Rhomboids 2 and 3, which are localized to both ER and late compartment. Tribolium ligand and chaperone behave in cell culture similar to the Drosophila counterparts, in terms of ligand trafficking and processing. However, the Tribolium chaperone, Star, is not cleaved by Rhomboids. This makes the system oblivious to the ER activity of Rhomboid, to produce long-range signals. The mechanistic basis for the non-cleavability of Tribolium Star is currently under examination.

Closing the GAP of molecular switches – a question of specificity

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Small GTPases of the Rho/Rac family are highly conserved proteins that play important roles in the spatio-temporal regulation of many cellular processes. They act as molecular switches in complex signalling cascades. In the off-state small GTPases are bound by guaninnucleotide exchange factors (GEFs) and activated by the release of GDP and the uptake of GTP. In the on-state they interact with downstream effectors, which regulate cell growth, cell polarity and cell separation.
In Ustilago maydis we could identify 6 Rho/Rac-like GTPases (1), which are involved in cell separation after cytokinesis (Cdc42), in polar growth (Rac1) and, putatively, in cell wall synthesis (Uro1). The functions of the other three Rho proteins Rho2, Rho3 and Rho4 have not been analysed so far.
To avoid nonsense activity of small GTPases during the cell cycle, cells express GTPase activating proteins (GAPs), which stimulate the intrinsic GTPase activity of small GTPases to hydrolyse GTP to GDP followed by inactivation of the GTPases. After genomic mining we have identifed 10 putative GAP proteins in the U. maydis database, for which we have started to determine specificity.
I will present my results of a) knock-out strains and their phenotypes, b) overexpression of the GAP domains and the clues we draw for specificity, c) localisation of full length proteins in haploid cells and dicaryotic filaments and d) in vitro GAP-assay to verify specificity.

Regulation of cytokinesis by the Rho guanine nucleotide exchange factor Don1 in Ustilago maydis

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Small GTP-binding proteins of the Rho/Rac family are molecular switches that regulate a plethora of cellular functions, including polarity determination, cytoskeletal reorganisation and membrane trafficking. We are studying the molecular function of Rho/Rac-GTPases, especially Cdc42 and Rac1, in the dimorphic fungus Ustilago maydis. Deletion mutants of Cdc42 are viable but show a cell separation defect. Deletion mutants of Rac1 are also viable but are defective in polarized growth. We have identified activators of both Cdc42 and Rac1. The small GTPase Rac1 is specifically activated by the guanine nucleotide exchange factor Cdc24. Overexpression of this GEF leads to Rac1-dependent polarized growth. In vitro GDP/GTP exchange assays show that Cdc24 can also activate Rac1 in vitro.
Cdc42 is activated by the guanine nucleotide exchange factor (GEF) Don1, a key regulator of cytokinesis in U. maydis. Using in vitro GEF assays, we could demonstrate that Don1 acts as guanine nucleotide exchange factor for Cdc42 but cannot activate the closely related GTPase Rac1. However, Rac1 with a single amino acid from Cdc42 is a substrate for Don1 both in vitro and in vivo.
Don1 belongs to the FGD1 family of Cdc42-specific GEFs that are characterized by a C-terminal lipid-binding FYVE domain. Although the FGD1 family of Rho-GEFs is evolutionary conserved from fungi to mammals the role of the FYVE domain for GEF function is unknown. We could show that the FYVE domain is critical to target Don1 to the site of septation. This localization is essential to trigger cytokinesis. We could demonstrate that the Don1 FYVE domain binds to PtdIns(3)P in vitro and to endosomal membranes in vivo. We found that the FYVE domain mediates binding of Don1 to PtdIns(3)P-rich endosomal vesicles that are transported to the site of septation zone in a Kinesin-dependent fashion. Kinesin-driven accumulation of endosomal vesicles at the division site is not only required for localized Don1 activity but is also prerequisite for proper lysis of the connecting cell wall. Our results demonstrate how the coordination of cytokinesis and cell abscission is achieved by compartmentalization of a Cdc42-specific GEF.

Bicaudal-D Like Protein Madmax1 Organizes Dynein-dependent Secretory Transport And Neural Development

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Membrane and secretory trafficking is essential for proper cellular development. However, the molecular mechanisms that organize secretory trafficking are poorly understood. Here, we identify Bicaudal-D like protein Madmax-1 as an effector of Rab6 and key component of the molecular machinery that coordinates dynein-dependent secretory trafficking. We show that Madmax-1 interacts with the dynein-dynactin retrograde motor complex, recruits Rab6-positive secretory vesicles to the centrosome, organizes secretory vesicle exocytosis at the plasma membrane and is required for neurodevelopment in zebrafish. In hippocampal neurons, Madmax-1 expression strongly decreases during neurite outgrowth. During early stages of development Madmax-1 accumulates dynein-dynactin and secretory vesicles in the neuronal cell body while later reduced Madmax-1 levels allow for secretory transport into developing neurites. Persistent expression of Madmax-1, as well as knockdown of both Rab6A and Rab6B suppresses neurite outgrowth. These results indicate an important role for Madmax-1 in neural development by serving as an organizer for dynein-dependent trafficking of secretory vesicles. The data uncover a new mechanism to achieve regulation and specificity in secretory trafficking and exocytosis during neuronal development.

SPARC is required for Notch signaling during the development of Drosophila Melanogaster

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Extracellular matrix (ECM) molecules are multifunctional secreted proteins that play a crucial role in animal development and morphogenesis. Many ECM molecules have structural functions, endowing tissues with tensile strength as well providing a scaffold for cell adhesion and migration. However, numerous ECM molecules also have intracellular functions independent of their extracellular structural contributions and can play an active role in cell signalling, differentiation, polarization and survival.

Sparc (Secreted Protein Acidic Rich in Cysteine) is a calcium binding extracellular matrix (ECM) glycoprotein whose trimodular organization is evolutionarily conserved between arthropods and vertebrates. Despite decades of research, its precise functions during embryogenesis and development are poorly understood.

In the Drosophila embryo, anti-Sparc immunostaining shows that it is a major component of basal laminae, colocalizing extensively with Collagen IV and Laminin in the ECM. Immunostaining for Sparc also shows a ubiquitous intraceullar punctuate localization reminiscent of endocytic and exocyst compartments. Sparc null mutants are embryonic lethal with severe developmental defects in most tisssues examined. Analysis of nervous and tracheal system development in Sparc null mutants revealed that Notch signalling is disrupted in the absence of Sparc. This phenotype is enhanced by the loss of Dynamin, Rab5 and Sec15 indicating that Sparc may be functioning to regulate intracellular trafficking or endocytosis of Notch or it's ligand Delta during lateral inhibition and cell specification. By using high throughput screens and the power of Drosophila genetics for structure function studies and clonal analysis, we are currently trying to elucidate the molecular mechanisms by which Sparc is acting to promote Notch signalling.

Anterior embryonic polarity is maintained by dynamin

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Cell polarity is crucial for the generation of cell diversity. The PAR proteins and the actin cytoskeleton play central roles in generating and maintaining cell polarity in many systems (Betschinger, 2004; Munro, 2006), yet, the mechanisms that maintain polarity cues in particular membrane domains during development remain elusive. A genome-wide RNAi screen in C. elegans recently revealed that anterior polarity cues could specifically regulate the endocytic machinery (Balklava, 2007), and current models suggest that PAR-dependent membrane recycling may maintain membrane domains and boundaries throughout development (Wissler, 2007). However, the links between the PAR proteins, endocytic machinery and the actin cytoskeleton during development are unknown.

Dynamin is a large GTPase that plays key roles in both endocytosis and actin dynamics and therefore, a potential factor that could connect these events to cell polarity pathways. Here, we show that C. elegans dynamin, DYN-1, is required to maintain the anterior polarity factors PAR-6, RHO-1 and CDC-42 as well as the spindle pole movements in the developing embryo. Interestingly, DYN-1-GFP foci are enriched in the anterior cortex of the embryo where endocytosis primarily occurs. PAR-6-labeled foci are closely associated with RAB-5 foci, EEA-1, and FM1-43-labeled vesicles, suggesting that PAR-6 is endocytosed and recycled back to the anterior cortex during maintenance phase. FRAP experiments reveal a significant difference in PAR-6 dynamics between polarity establishment and maintenance phases, supporting a model in which a dynamic mechanism is involved in restricting anterior polarity cues to their appropriate domain. DYN-1 regulates anterior membrane and actin comet dynamics specifically during the polarity maintenance phase. Foci of actin closely associated with RAB-5 foci, suggesting that actin-based endocytosis occurs in the one-celled embryo. Our results demonstrate a dynamin-dependent mechanism for the spatial and temporal regulation of polarity, endocytosis and actin dynamics in the anterior of the embryo, contributing to the precise localization and maintenance of polarity factors within a dynamic plasma membrane.

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Poster 92

Na+,K+-ATPase POLARITY; the role of the beta-subunit

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A century-and-a-half ago Du Bois Raymond found that epithelia can maintain an electrical potential across. The explanation had to wait another century, until J.Ch. Skou discovered the Na+,K+-ATPase, and Koeffoed-Johnson & Ussing (KJ-U) put forward a model to account for the electrical potential, as well as the vectorial movement of Na+ . An essential assumption of the KJ-U model was that the Na+,K+-ATPase is polarized to the basolateral side. The plausible explanation for this polarization took another half-a-century. The main problem was that, contrary to the polarity of most membrane proteins, the Na+,K+-ATPase has no sorting signal nor a retention sequence. Five clues enabled us to solve this problem: (1) The alpha and beta subunits are firmly bound to each other. (2) The beta one has the characteristics of a cell- attaching molecule1. (3) Epithelial cells only express the enzyme at a given border provided that the neighboring cell also contribute with its own2. (4) As a corollary, an epithelial cell does not express their Na+,K+-ATPase at the basolateral border (as originally proposed by KJ-U) but just at the lateral one2,3. (5) The expression is species-specific. By transfecting dog beta-subunit to CHO cells, we demonstrated that the polarized distribution of Na+,K+-ATPase is due to beta-beta recognition across the intercellular space3 and by protein-protein interaction assays we show that beta-beta interaction anchors the whole enzyme in this position4.

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Anterior embryonic polarity is maintained by dynamin

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Cell polarity is crucial for the generation of cell diversity. The PAR proteins and the actin cytoskeleton play central roles in generating and maintaining cell polarity in many systems (Betschinger, 2004; Munro, 2006), yet, the mechanisms that maintain polarity cues in particular membrane domains during development remain elusive. A genome-wide RNAi screen in C. elegans recently revealed that anterior polarity cues could specifically regulate the endocytic machinery (Balklava, 2007), and current models suggest that PAR-dependent membrane recycling may maintain membrane domains and boundaries throughout development (Wissler, 2007). However, the links between the PAR proteins, endocytic machinery and the actin cytoskeleton during development are unknown.

Dynamin is a large GTPase that plays key roles in both endocytosis and actin dynamics and therefore, a potential factor that could connect these events to cell polarity pathways. Here, we show that C. elegans dynamin, DYN-1, is required to maintain the anterior polarity factors PAR-6, RHO-1 and CDC-42 as well as the spindle pole movements in the developing embryo. Interestingly, DYN-1-GFP foci are enriched in the anterior cortex of the embryo where endocytosis primarily occurs. PAR-6-labeled foci are closely associated with RAB-5 foci, EEA-1, and FM1-43-labeled vesicles, suggesting that PAR-6 is endocytosed and recycled back to the anterior cortex during maintenance phase. FRAP experiments reveal a significant difference in PAR-6 dynamics between polarity establishment and maintenance phases, supporting a model in which a dynamic mechanism is involved in restricting anterior polarity cues to their appropriate domain. DYN-1 regulates anterior membrane and actin comet dynamics specifically during the polarity maintenance phase. Foci of actin closely associated with RAB-5 foci, suggesting that actin-based endocytosis occurs in the one-celled embryo. Our results demonstrate a dynamin-dependent mechanism for the spatial and temporal regulation of polarity, endocytosis and actin dynamics in the anterior of the embryo, contributing to the precise localization and maintenance of polarity factors within a dynamic plasma membrane.

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Poster 94

Kinetic characterization of clathrin cage disassembly by the molecular chaperone Hsc70 and its partner auxilin

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Clathrin mediated endocytosis is a fundamental function of eukaryotic cells. It is important for nutrient uptake, protein trafficking, cell signalling and development. In a coordinated process involving many different proteins, a clathrin-coated invagination is formed on the cytoplasmic face of the plasma membrane, which eventually buds off to form a clathrin coated vesicle carrying protein cargo. Before the vesicle can fuse with its intracellular target membrane the cage has to be removed. This uncoating of clathrin coated vesicles is achieved by the chaperone protein Hsc70 and its partner auxilin, in an ATP-dependent process.
Using purified protein components and biophysical methods our aim is to examine the mechanism of clathrin cage disassembly by Hsc70 and auxilin, to determine the kinetics of the disassembly, and dissect the function of the individual components.
Clathrin is composed of a heavy chain and a light chain. Three clathrin molecules come together to form a ‘three-legged’ triskelion. In vitro, these triskelions can form cages in the absence of lipid. Using a novel assay based on light scattering, we have shown that disassembly occurs quickly, with a maximal rate of 0.065 s-1 (t1/2 ≈ 10 s). Whilst excess Hsc70 is required for the maximal rate of disassembly, auxilin is only required at a ratio of one per triskelion (three clathrin molecules). ATP hydrolysis is required not only for the cage disassembly but for the clathrin:auxilin:Hsc70 complex to form, and ATP is hydrolysed during the disassembly process at a rate of 1 mol per mol of clathrin disassembled. These results, together with previously published data, suggest that a single auxilin bound to three clathrin molecules (or one triskelion) somehow recruits three Hsc70 molecules which each hydrolyse one ATP during the disassembly process. Yet isothermal titration calorimetry shows that Hsc70 and auxilin bind at a ratio of 1:1 in the absence of clathrin. Future work aims to examine the interactions using fluorescence anisotropy, to try to understand the mechanism underlying auxilin’s recruitment of Hsc70, and how Hsc70 removes a clathrin triskelion from a cage.

Understanding enterovirus interactions with human polarized epithelial cells and the subsequent cellular processes

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Enteroviruses are though to initiate their infectious cycle by a productive infection of the intestinal epithelium but the interaction of these viruses with polarised epithelial cells is poorly understood. The glycosylphosphatidylinositol (GPI)-anchored complement regulatory protein-accelerating factor (DAF or CD55) is used as receptor by echovirus during the early stages of infection. DAF and other GPI-anchored proteins are found in cholesterol-rich ordered micro-domains within the apical membrane of polarised epithelial cells that are known as “lipid rafts”. Here, we report that echovirus 11 (strain 207) initiates infection by binding to it’s receptor at the apical membrane of polarised caco-2 cells. The virus replicates within 4 hours post-infection (p.i). At 6 p.i hours the virions are released through the apical surface. Following the binding to the receptor, echo11 is rapidly transferred to tight junctions. We show that this rapid transfer of the virus to tight junctions is dependent on actin and rafts. Further we found that internalization of echo11 from tight junctions is tyrosine kinases dependant.

Dissection of Drosophila STAT to find new functional domains related to its polarized signalling.

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The JAK/STAT signalling pathway is involved in processes ranging from immune response to organogenesis. We recently showed that JAK/STAT signalling in the Drosophila ectoderm is strongly influenced by the cell's polarity.
In contrast to the conventional vertebrate signalling model based on cell culture experiments where the receptor does not localise to any particular domain and the inactive STAT is in the cytoplasm, we showed that the receptor, JAK kinase and STAT protein localize apically in the ectoderm. STAT's apical localization is dependent on the polarity protein PAR-3 and this apical localization is required for efficient signalling. To find out what domains of STAT are required for the apical localization and function, we are dissecting the protein and analyzing the function of the different domains in vivo. Using immunoprecipitation assays we are also analyzing what domains bind directly to PAR-3. We are also studying Xenopus STAT proteins to find out if polarized apical localization is a general feature also occuring in vertebrates.

Centriole-Mediated, Minus End-Directed Transport of Lytic Granules to Secretory Sites during Target Cell Killing by Cytotoxic T-lymphocytes

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Cytotoxic T-lymphocytes (CTLs) kill virally infected and tumourogenic cells by releasing potent lytic material which causes apoptosis in targeted cells. To prevent innocent cell death, lytic material is stored in lysosomes (termed 'lytic granules' (LG)) and released only on contact with a target; at the site of contact; into a sealed space or 'secretory cleft' between the two cells. This regulation is mediated by polarisation events which are initiated in CTL by target cell contact, including: 1) rearrangement of plasma membrane proteins at the contact site to a) establish the cleft and b) form functionally distinct membrane domains (including a specialised 'secretory domain' opposite the cleft); 2) clearance of actin from the contact site; 3) MTOC polarisation and reorientation of the microtubule cytoskeleton towards the contact site; 4) polarised LG delivery to the secretory domain. These events are transient and reverse on completion of killing.
We are interested in mechanisms involved in CTL polarisation, particularly the targeted transport and secretion of lytic material. We recently showed that during killing CTL centrioles move right to the plasma membrane and contact it at the edge of the secretory domain. This localisation aligns microtubules under the plasma membrane at the secretory domain meaning LG are both directed and delivered to the secretory site in a single step during minus (centriole) end-directed transport. CTL therefore use a novel mechanism for LG delivery and differ from both conventional secretory cells (which show plus end-directed transport of organelles to secretory sites) and most tissues showing MTOC polarisation (where reorientated centrioles remain within the cell body).
We are currently taking high resolution morphological (EM) and biochemical approaches to dissect the mechanism of centriole-mediated minus end-directed transport. We find that other immune cells which kill targets by polarised secretion from LG, e.g. Natural Killer (NK) and NKT-lymphocytes, also use this mechanism, suggesting a specialised mechanism common to lytic haemopoietic cells. Using normal CTL, CTL from patients with genetic diseases affecting killing, or CTL where potential mechanistic proteins have been removed we have been 1) further characterising the behaviour and organisation of centrioles during polarisation and 2)identifying proteins involved at different stages in centriole-mediated minus end-directed LG delivery. This has allowed us to identify distinct stages of the centriole polarisation and LG deliver pathways, and map particular proteins to specific steps. Together our results further our understanding of the molecular mechanisms involved in polarised LG exocytosis.

Reggie-1 and reggie-2 (flotillins) regulate axon growth and regeneration

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The reggies/flotillins - proteins upregulated during axon regeneration in retinal ganglion cells (RGC) - are scaffolding proteins of microdomains and involved in neuronal differentiation. Here, we show that reggies regulate axon regeneration in zebrafish (ZF) after optic nerve section (ONS) in vivo as well as axon/neurite extension in hippocampal and N2a neurons in vitro through signal transduction molecules modulating actin dynamics. ZF reggie-1a, -2a and -2b downregulation by reggie-specific morpholino antisense (Mo) oligonucleotides directly after ONS significantly reduced ZF RGC axon regeneration: RGC axons from reggie Mo-retinae were markedly reduced. Moreover, the number of axon-regenerating RGCs, identified by insertion of A488-coupled dextran, decreased by 69% in retinae 7d after Mo-application, and by 53 and 33% at 10 and 14d, respectively, in correlation with the gradual degradation of the Mos. siRNA-mediated knockdown of reggie-1 and -2 inhibited the differentiation and axon/neurite extension in hippocampal and N2a neurons. N2a cells had significantly shorter filopodia, more cells had lamellipodia and fewer neurites, defects which were rescued by a reggie-1 construct without siRNA binding sites. Furthermore, reggie knockdown strongly perturbed the balanced activation of the Rho family GTPases Rac1, RhoA and cdc42, blocked the phosphorylation of cortactin and the formation of the cofilin, N-WASP, Arp2/3 complex, and affected p38, Ras, ERK1/2 and focal adhesion kinase (FAK) activation. Thus, as suggested by their prominent re-expression upon lesion, the reggies represent neuron-intrinsic factors for axon outgrowth and regeneration, being crucial for the coordinated assembly of signalling complexes regulating cytoskeletal remodelling.

Repositioning of the centrosome and Golgi complex during the transition from apical to basal progenitors in mouse embryonic neocortex

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During mammalian embryogenesis, cortical neurons arise from divisions of neural progenitors. In the embryonic cortex, two classes of neural progenitors have been identified: (i) neuroepithelial (NE) cells dividing at the apical side of the ventricular zone (VZ), also referred to as apical progenitors (APs) and (ii) progenitors dividing at the basal side of the ventricular zone, also referred to as basal progenitors (BPs). Notably, apical progenitors show apical-basal polarity and span the entire cortical wall, with an apical membrane lining the ventricle and a basal end-foot at the pial surface. Basal progenitors lose apical-basal polarity by delaminating from the apical surface, their apical process being retracted before the onset of mitosis. To further investigate the reorganisation of cell polarity during the transition from APs to BPs, we focus our attention on the Golgi complex. In apical progenitors, the Golgi complex spans the entire ventricular zone. Interestingly, the Golgi complex is not organised around the centrosome, with the distance between the centrosome and the apical-most part of the Golgi ranging from 3 to 20 ?m. In addition, although not pericentrosomal, the Golgi complex is undergoing disassembly during mitosis. Finally, in basal progenitors, the position of the centrosome and Golgi complex correlates with delamination, with the Golgi complex becoming pericentrosomal by the end of the delamination process.

Sds22, a PP1 phosphatase regulatory subunit, regulates epithelial cell polarity and shape

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Background: How epithelial cells adopt their particular polarised forms is poorly understood. In a screen for genes regulating epithelial morphology in Drosophila, we identified sds22, a conserved gene previously characterised in yeast. Results: In the columnar epithelia of imaginal discs or follicle cells, mutation of sds22 causes contraction of cells along their apical-basal axis, resulting in a more cuboidal morphology. In addition, the mutant cells can also display altered cell polarity, forming multiple layers in follicle cells and leaving the epithelium in imaginal discs. In yeast, sds22 encodes a PP1 phosphatase regulatory subunit. Consistent with this, we show that Drosophila Sds22 binds to all four Drosophila PP1s and shares an overlapping phenotype with PP1beta9c. We also show that two previously postulated PP1 targets, Spaghetti Squash and Moesin are hyper-phosphorylated in sds22 mutants. This function is shared by the human homologue of Sds22, PPP1R7. Conclusions: Sds22 is a conserved PP1 phosphatase regulatory subunit that controls cell shape and polarity.

TIRF microscopy for studying apical membrane trafficking

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In total internal reflection fluorescence (TIRF) microscopy an evanescent wave is generated by the illuminating laser beam that penetrates approximately 100nm into the region beyond the glass cover slip. Thereby this method allows for monitoring motion and membrane fusion of vesicles carrying membrane proteins with high temporal and spatial resolution by efficiently suppressing signals coming from regions deeper within the cell. Because of optical prerequisites necessary to achieve total internal reflection, TIRF microscopy so far was limited to study membrane trafficking at the basolateral membrane at sites where cells are attached to the glass cover slip.
We overcome this limitation by moving cell monolayers towards a glass cover slip in a controlled manner, such that they dive with their apical membrane into the region illuminated by the evanescent wave. The controlled approaching of a cell monolayer was realized via a microfluidic biochip with an integrated positioning system. The chip was applied to visualize fluorescently tagged aquaporins as markers of the apical membrane in Madin-Darby canine kidney (MDCK) cells by TIRF microscopy. With a combination of TIRF microscopy and the fluorescence recovery after photobleaching (FRAP) technique we studied the refilling dynamics after selectively bleaching the apical membrane.

Clathrin Modulates Membrane Localization of the Heterotrimeric G Protein Subunits Gβγ And Spindle Positioning in C. elegans Embryos

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Asymmetric cell division is a conserved mechanism used to generate daughter cells that possess distinct fates. Correct orientation of the mitotic spindle with respect to the polarity axis is crucial to this process. In the one-cell stage embryo of the nematode C. elegans, polarity cues define an anterior-posterior axis shortly after fertilization, and the first division occurs perpendicular to this axis. Moreover, the mitotic spindle is positioned asymmetrically towards the posterior of the embryo, resulting in daughter cells that differ not only in fates, but also in sizes. Enrichment of active cortical force generators at the posterior compared to the anterior is at the root of asymmetric spindle positioning. Cortical force generating complexes comprise two redundant Gα proteins (GOA-1 and GPA-16), the GoLoco domain containing proteins GPR-1/2 and the coiled-coil domain protein LIN-5.The current working model postulates that these complexes serve to anchor the motor protein dynein to the cell cortex, thus allowing the generation of pulling forces on the plus ends of microtubules that reach out from the asters. Pulling forces are in turn negatively regulated by the heterotrimeric G protein Gβγ subunits GPB-1 and GPC-2, presumably by restricting the availability of the Gα proteins for interaction with GPR-1/2 and LIN-5. Although the way by which force is generated is becoming increasingly well understood, the mechanisms by which polarity cues regulate the asymmetric distribution of active cortical force generators remains an open question. We found that depletion of the clathrin heavy chain CHC-1 from early embryos using RNAi leads to increased forces during spindle positioning. Furthermore, the Gβγ subunits are mislocalized in chc-1(RNAi) embryos. Whereas GPB-1 and GPC-2 are present at the plasma membrane in wild type, colocalization experiments revealed that GPB-1 resides in RAB-7 positive endosomes in chc-1(RNAi) embryos. Furthermore, depletion of other endocytic regulators such as RAB-5 and dynamin similarly alters GPB-1 localization and pulling forces in the one-cell stage embryo. Recent reports of asymmetric anterior enrichment of select endocytic compartments (early and recycling endosomes), together with our own observations suggest that, asymmetric intracellular trafficking of key modulators of spindle positioning might be crucial for proper asymmetric spindle positioning in C. elegans.

Efficient coupling of Sec23/24 to Sec13/31 is required for collagen secretion and expansion of the lumen in Caco-2 cell cysts

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The COPII coat is required for the majority of protein and lipid transport from the endoplasmic reticulum (ER) to the Golgi. Initiation of COPII coat assembly is through GDP/GTP exchange of a small GTPase, Sar1, by its guanine nucleotide exchange factor Sec12. Sar1-GTP embeds into the ER membrane where it recruits Sec23/24, a heterodimeric complex. Sec23 is a GTPase activating protein for Sar1 and Sec24 is responsible for the majority of cargo capture. Sec13/31, a heterotetrameric complex is then recruited. This forms the structural cage around the budding vesicle and binding of this outer layer causes formation of the COPII coat which is then rapidly dissociated to reveal the mature vesicle. We show that suppression of Sec13/31 using RNA interference in human primary fibroblasts caused a defect in the transport of procollagen. In contrast, export of tsO45-G-YFP and GalT from the ER was unaffected suggesting that small, more freely diffusible cargo was still able to be secreted. Electron microscopy of high pressure frozen cells showed that the ER was distended in Sec13/31 depleted cells and decorated with large, apparently coated, open-necked budding profiles. Morpholino-based suppression of Sec13/31 in zebrafish caused defects in craniofacial development and proteoglycan deposition. These data suggest that transport of large extracellular matrix proteins such as collagen require sufficient levels of Sec13/31 for full assembly of the COPII coat. These and other observations led us to examine the consequences of Sec13/31 suppression for epithelial cell polarization and differentiation. We have used Caco-2 cells in which expression of Sec13/31 has been stably suppressed to examine the consequences for epithelial differentiation and polarization. Cells grown on non-coated permeable supports show disorganization of the epithelial layer, while those grown as 3D cysts within tumour-derived basement membrane matrix show less disorganization but are defective in lumen expansion. Possible defects in cell polarity and differentiation are now under investigation. These data suggest a key role for COPII-dependent trafficking from the ER in the differentiation of polarized cells.

Control of Symmetric versus Asymmetric Cell Division in a C. elegans Epithelium

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Polarized cells may divide symmetrically or asymmetrically. This choice has major consequences, as asymmetric division leads to the generation of daughter cells with different fates and/or life span. In addition, symmetric division promotes an exponential increase in the number of dividing cells, while this number can be stable through many cycles of asymmetric division. The importance of this mechanism is illustrated by stem-cell like progenitors that combine self-renewal with the generation of more differentiated daughters for many years.
As an attractive model for asymmetric cell division, we explore the C. elegans epidermal “seam” cells; a polarized epithelium with stem-cell like divisions. Cells in this lineage go through symmetric and asymmetric divisions at completely reproducible times of development. Hence, genetic analysis of the seam lineage will help define which genes act critically in the control of symmetric versus asymmetric division. We have created a number of transgenic reporter strains in which key components of the division process are marked with fluorescent tags in the epidermal seam cells. This allows us to follow the position of the centrosomes and spindle, chromosomes, cell junctions, and cell membranes in wild-type and mutant animals by time-lapse fluorescence microscopy.
Our observations have revealed how the mitotic spindle forms and positions along the anterior-posterior axis during symmetric and asymmetric divisions of the seam cells. Regulators of spindle positioning in the early embryo, such as the NuMA/Mud related protein LIN-5, contribute to this process. Starting in the second larval stage, the spindle becomes anteriorly displaced during anaphase. Consequently, cell division generates a smaller anterior and larger posterior daughter. Our preliminary results indicate that Wnt signaling, rather than a typical planar cell polarity pathway, controls this asymmetry. Finally, we have identified contributions at several additional levels -junction associated tumor suppressors, a miRNA pathway, the general cell-cycle machinery- in the control of the proper cleavage pattern. Ultimately, quantitative studies of the C. elegans seam cell lineage will help characterize the regulatory network for division control in a polarized epithelium.

Apical recycling endosome-associated myosin Vb is mutated in microvillus inclusion disease and is involved in intestinal brush border development

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A structurally, compositionally, and functionally distinct plasma membrane at the apex of the intestinal epithelial cell monolayer provides a selective and protective barrier that regulates the uptake of nutrients from the lumen. The inability of intestinal cells to develop an apical brush border and the consequences it has on human functioning becomes particularly apparent in patients diagnosed with microvillus inclusion disease (MID), a rare hereditary enteropathy presenting with severe intractable diarrhea and malabsorption in neonates. At the cellular level, brush border atrophy with accumulation of lysosomal granules and microvillus inclusions is observed in the apical cytoplasm of MID enterocytes. Apical brush border components are typically absent from the cell surface and accumulate in the apical cytoplasm. In contrast to the apical proteins, basolateral proteins display a normal polarized distribution at the surface of MID enterocytes, which appear normally arranged in monolayers with distinguishable cell-cell adhesion junctions. Because of the specific loss of apical surface identity, MID provides an outstanding opportunity to study the genetics and molecular dynamics that underlie apical surface development.
Defective intracellular trafficking of apical, brush border proteins in MID has been proposed. The exact nature of such impairment remains obscure but may occur in the biosynthetic pathway via which newly synthesized brush border proteins are delivered from the trans-Golgi network to the cell apex, or in the apical recycling route via which brush border proteins are recycled back to this surface domain. The small GTPase Rab11a and a family of Rab11a-interacting proteins that also includes myosin Vb regulate apical recycling in cultured epithelial cells. Furthermore, apical recycling endosome dynamics have been implicated in the biogenesis of apical surface domains, although direct in vivo evidence for such a relationship has not been presented thus far.
In this study, we have identified mutations in the MYO5B gene in MID patients. MYO5B encodes for an apical recycling endosome-associated and actin filament-binding molecular motor protein. We also found aberrant expression and subcellular distribution of myosin Vb and other key proteins that interact with myosin Vb and/or control apical recycling endosome-mediated protein trafficking, and an increase in appearance of late endosomes and/or lysosomes.
Taken together, the endosomal system that ensures the recycling of brush border proteins, with myosin Vb as a critical regulator, is required to develop the apical cell surface in human enterocytes, and perturbations in this can be causally linked to microvillus inclusion disease.

Role of retromer in traffic of the beta-site amyloid precursor protein (APP) cleaving enzyme (BACE) and beta-APP

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Retromer is an evolutionary conserved protein complex that mediates endosome-to-Golgi retrieval of receptors for lysosomal hydrolases. It is constituted by five Vacuolar protein sorting (Vps) subunits organized into two sub-complexes: one formed by Vps26-Vps35-Vps29, in charge of cargo recognition, and another one comprising the two sorting nexins SNX1 and SNX2, which deforms the membrane to ensure cargo sorting. Research in different organisms indicates that retromer participates in the sorting of additional proteins. Two of such putative cargos are the amyloid precursor protein (beta-APP) and the beta-site APP cleaving enzyme (BACE or beta-secretase), which hydrolyzes beta-APP leading to production of the neurotoxic amyloid beta peptide and progression of Alzheimer disease. Here, we study the connection between retromer and BACE-APP traffic in two models of polarized cells, neurons and Madin-Darby canine kidney (MDCK) cells, which share some mechanisms for polarized sorting. Immunohistochemical detection of retromer subunits in adult mouse brain revealed a similar pattern of regional distribution, including neurons of the cerebral cortex, hippocampus (pyramidal cells), basal ganglia, substantia nigra, striatum and cerebellum. In cerebellum, an inside-out gradient of retromer expression was observed, with apparent staining in dendrites of Purkinje cells but not in their axons. In primary cultures of neurons differentiated in vitro from subventricular zone neurospheres, ectopically expressed BACE partially co-localized with Vps26 in the soma as well as in vesicles traveling along neurites. The use of MDCK cells as a model system has allowed the generation of cell lines stably co-transfected with BACE and APP. Co-immunoprecipitation of Vps35 with myc-BACE and APP suggests an interaction of retromer with these proteins in vivo. Treatment of MDCK cells with the phosphoinositide 3-kinase (PI3K) inhibitor LY294002, which affects retromer's function, alters the association between myc-BACE and APP. Inhibiting PI3K, localization of BACE in endosomes is increased, suggesting its retention in endosomes in the absence of retromer's function. These results support a role of retromer in traffic of BACE and/or APP.

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Function of ciliary gene RPGRIP1L/Ftm during zebrafish development.

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We have undertaken the cloning of the orthologue of RPGRIP1L/Ftm in zebrafish to assess its function in zebrafish. Injection of 2 different morpholinos against either the ATG region or within the 5'UTR leads to the same phenotype: embryos that present a shorter tail with undulated notochord. The defect in convergence-extension can be measured by the « body gap angle » and was shown to be dose-dependent and specific since it can be rescued by the injection of the human Ftm RNA, not targeted by the morpholino. Because we observe a convergence-extension phenotype, we have tested if the Wnt/PCP pathway interacts genetically with Ftm by injecting in embryos heterozygous for a Vangl2 mutation. At this low dose, no defect is detected in wild-type embryos while a strong phenotype is observed in 50% of the embryos that are Vangl2 heterozygous. We are now trying to rescue Ftm morphants gastrulation phenotype by injection of active components of the Wnt-PCP pathway.
Because several components of this pathway have been described at the base of the primary cilium like the Ftm protein, we are assaying their sub cellular localization as well as their abundance in the Ftm morphants. One function of Ftm could be to regulate their traffic to the cilium or their function at this specific location.
Another aspect of Ftm morphants phenotype is a left-right laterality defect that can be traced back to either an incorrect specification of the precursors of the zebrafish laterality organ, the dorsal forerunners cells in morphants with no Küppfer vesicle or to impaired ciliogenesis for morphants bearing a slightly smaller Küppfer vesicle. We are now characterising Ftm molecular partners to perform these 2 different functions.

Roles of IQGAP1 in regulation of microtubule dynamics in polarized migrating cells

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Polarized cell migration is required for various cell behaviors and functions. Microtubules are structurally coupled to actin filaments and distributed asymmetrically along the front-rear axis of migrating cells to ensure biased vesicular transport for maintenance of cell polarity. +TIPs (Plus-ends-tracking proteins) affect microtubule dynamics and play important roles in coupling of microtubules to actin filaments. The typical +TIPs such as EB1 and CLIP-170 accumulate at growing ends of microtubules uniformly. Among +TIPs, adenomatous polyposis coli (APC) and CLASPs asymmetrically accumulate at some population of growing ends of microtubules near the cortical regions. Thus, they are likely involved in a region-specific control of microtubules in polarized migrating cells. At the front leading edges, small GTPases Rac1 and Cdc42 regulate the rearrangement of adhesion and organization of cytoskeleton to establish cell polarity during directional migration. Growing evidence shows that Rho family GTPases affect the dynamics of microtubules through their effectors such as PAK, Par complex, and IQGAP. IQGAP1, an effector of Rac1 and Cdc42, binds to and cross-links actin filaments. In migrating fibroblasts, IQGAP1 accumulates at the front leading edges where microtubules and actin filaments are regionally coupled. We have found that IQGAP1 interacts directly with CLIP-170 and CLASP2. Elimination of IQGAP1 impairs CLIP-170 immobilization at the leading edges, but does not the ends tracking of CLIP-170 and CLASP2. Activated Rac1/Cdc42 and GSK-3 regulate the association of IQGAP1 with those +TIPs. We are currently investigating the roles of IQGAP1 in microtubule regulation in cell-free system. Using purified +TIPs and IQGAP1, we are reconstituting the connection of microtubule to the cortex. Preliminary results show that microtubules are guided along the IQGAP1-coated wall only in the presence of CLIP-170. This approach would shed light on the novel mechanisms how asymmetric distribution of microtubules is established during cell polarization. I would like to talk about our latest results from the reconstitution of microtubule dynamics in cell-free system.

Different requirements for incorporation of GPI-anchored proteins into ER-derived vesicles in yeast and mammalian cells

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GPI-anchored proteins and transmembrane proteins are incorporated into different populations of ER-derived vesicles in yeast. In order to address whether GPI-anchored proteins are incorporated into different vesicles from transmembrane proteins in mammalian cells, we reconstituted ER-derived vesicle formation in vitro using radio labeled microsome fractions or semi-intact cells from CHO cells. Using in vitro produced vesicles, we analyzed distribution of the cargo proteins among vesicle fractions. Here we show that in mammalian cells, the GPI-anchored proteins, CD59 and Folate receptor, and several transmembrane proteins including VSV-G and Kit ligand are only partially segregated upon incorporation into ER-derived vesicles. Furthermore, ER-derived vesicle fractions containing GPI-anchored proteins and transmembrane proteins tether and fuse together to form a common ER-Golgi intermediate compartment. We are examining whether fluorescent protein-tagged transmembrane and GPI-anchored proteins accumulate in the same ER exit sites in vivo or not. The incorporation of GPI-anchored proteins into ER-derived vesicles is tightly dependent on functional Sar1 protein in mammalian cells, but to a much lesser extent in yeast cells in in vitro reconstitution assays. In yeast, it has been shown that concentration of GPI-anchored proteins prior to budding is less dependent on Sec12p and Sec16p compared to other transmembrane proteins, but requires lipid remodeling of the GPI-anchor. In contrast, the lipid remodeling of GPI-anchored proteins is thought to begin only after arrival to the Golgi compartment in mammalian cells. Taken together, our results demonstrate significant differences in the mechanisms of ER exit of GPI-anchored proteins, particularly concerning their concentration. The sites where GPI-anchored proteins and transmembrane proteins are sorted along the secretory pathway are also different in yeast and mammalian cells.

Defects in the secretory pathway induce supernumerary P-body formation at the ER through elevated intracellular Ca2+ levels

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During various cellular stresses, mRNA accumulates in cytoplasmic processing bodies (P-bodies) where it is sequestered and turned over. Here we report that the number of P-bodies significantly increased in mutants of the small GTPase Arf1 and various other secretory pathway mutants. Exposure of wild-type cells to cell wall or osmotic stress, but not oxidative stress or starvation, mimicked the strong increase in P-body formation observed in arf1 and secretory transport mutants. Interestingly, MAP kinase signaling through either the osmo-response or the cell wall integrity pathway could not relay the signal to induce P-body formation; rather changes in the intracellular Ca2+ levels caused an increase in P-body number. Moreover, cells grown in the presence of the Ca2+ chelator BAPTA strongly reduced P-body formation in arf1 and other secretory transport mutants. Importantly, P-bodies induced in arf1 mutants were indistinguishable from those formed under starvation conditions. In both cases, P-bodies appeared as spheres of about 40-100 nm adjacent to the ER, suggesting that translation and silencing/degradation occur in close proximity at the ER, independent of the kind of stress encountered. Our data indicates that upon elevated Ca2+ levels more mRNA needs to be sequestered and eventually, if these levels persist, turned over.

The Adaptor Protein Amot Mediates the loss of Apical Polarity by directly tethering Apical Polarity proteins to Trafficking Pathways

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The adapter protein Amot directly targets apical polarity proteins into a trafficking pathway to regulate the morphology of epithelial cells. Recent work has determined that the trafficking of apical polarity proteins is essential for controlling the identity of the apical domain. The adapter protein Amot encodes a novel domain that specifically binds membranes enriched in PIP and cholesterol. Thus, Amot re-orients the proteins to which it associates such as Patj, aPKC zeta, Par-3 and Mupp1 into a Clathrin independent trafficking pathway. Such targeting under normal circumstances is required for the maintenance of tight junctions and the apical PM. Under certain stimuli, the balance of targeting is shifted towards endosomes to effect the sequestration of apical polarity proteins away from the PM. This promotes the loss of the epithelial morphology and the induction of the migratory configuration.

STED nanoscopy in living cells using live cell compatible markers

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Stimulated Emission Depletion (STED) nanoscopy is a light microscopic technique offering a resolution far beyond the diffraction limit. In this approach, the excitation beam is overlayed with a doughnut-shaped, red-shifted STED beam, which deexcites the excited fluorophores before spontaneous fluorescence can occur. Since the deexcitation is only taking place in the outer region and not in the very center of the excitation spot, this gives rise to a diffraction-unlimited effective excitation. By scanning the nanosized excitation spot through the focus, images can be acquired with a sub-second frame rate, which is especially beneficial for live-cell imaging. Here we demonstrate the application of STED microscopy for imaging the interior of living mammalian cells and tissue. Utilizing a yellow fluorescent protein (YFP) to image individual structural elements of the endoplasmic reticulum (ER) and the tubular network revealed a focal plane (x,y) resolution < 50 nm inside the living cell, corresponding to a 4-fold improvement over that of a confocal microscope and a 16-fold reduction in the focal spot cross-sectional area. Time lapse STED imaging of dendritic spines of YFP-positive hippocampal neurons in organotypic slices outperforms confocal microscopy in revealing important structural details. As an alternative to the fluorescent protein we also employed a genetically encoded protein tag which can be labeled in vivo with modified organic dyes. To selectively label specific proteins, we utilized fusion proteins with the protein tag hAGT, which is commercially available as SNAP-Tag. hAGT reacts specifically and rapidly with benzylguanines carrying a fluorophore, leading to the covalent labeling of the fusion protein with the organic dye. We used the red-emitting dye tetramethylrhodamine (TMR) for selective labeling of hAGT fusions to connexin-43, a membrane protein forming gap junctions. A resolution of down to 40 nm in the focal plane was observed in living mammalian cells. Also, movies were obtained, showing the movement of connexin-43 clusters across the cell membrane with nanoscale resolution.

Thus diffraction-unlimited imaging of structures in the interior of living cells greatly expands the scope of light microscopy in cell biology.

Genome-wide RNAi screen in C.elegans intestine reveals PAR-5 to maintain apical recycling endosome positioning and apico-basal polarity of epithelial cells

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Epithelial cells define the interface between the internal and external milieu of virtually all multicellular organisms. Their structural and functional polarity comprises two biochemically distinct membrane surfaces, an apicobasally polarized cytoskeleton and a sophisticated membrane trafficking system. The apical recycling endosomes (ARE) contribute to maintain the distinctness of the apical and basolateral plasma membrane and of membrane organelles by recycling and sorting endocytosed membrane cargo. Their apically enriched steady state position is dependent on the microtubule cytoskeleton and constitutes a hallmark of the polarized organization of the membrane trafficking system in epithelial cells. Little is yet known about the molecular mechanisms that regulate the structural and functional properties of this important organelle.
In order to identify genes that couple the organization of membrane traffic to epithelial polarity maintenance, we conducted a genome-wide RNAi screen in the C.elegans intestine.
We generated a transgenic worm strain that expresses fluorescently tagged marker proteins, an apical membrane cargo to follow apical membrane traffic, and the small GTPase RAB-11 to label the ARE. These two apical markers allowed us identify genes that (1) function in the numerous routes in apical membrane traffic, (2) that maintain the spatial distribution and morphology of the ARE, and (3) that sustain the apicobasal polarity of epithelial cells.
As a reference readout to the apical cargo and ARE markers, we also analysed RNAi effects on the lysosomes of C.elegans, the autofluorescent lysosome-related organelles.
The distribution of organelles and cargo were monitored by automated acquisition of 2.2 million fluorescent images that were processed by an image analysis software that we developed. Out of 16,757 genes analyzed, about 400 mainly conserved candidates exhibited an altered marker distribution.
The most enriched group in this hit set represented membrane trafficking genes, underscoring the validity of our screening approach. After hierarchical clustering of phenotype profiles assigned to all candidates, 60 selected candidates were analyzed in various organelle-marker strains. These morphological secondary RNAi assays provided further insight into their roles in epithelial membrane traffic.

One of the candidates that we examined in greater detail is the conserved 14-3-3 protein PAR-5. Depletion of PAR-5 caused a particularly striking phenotype of ARE positioning and of the apicobasal polarity of the epithelial cells. Our data suggest a novel role of PAR-5 in coordinating the interplay of cytoskeletal organization and organization of the epithelial membrane traffic system in order to sustain apico-basal polarity.

The tumor suppressor Lgl controls the polarized localization of Fragile X protein and associated mRNAs in neurons

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Fragile X syndrome (FraX) is the most common form of inherited mental retardation and is caused by loss-of-function for the FMR1 gene. FMR1 encodes an RNA-binding protein (FMRP), thought to function in neural development and synaptic plasticity by controlling the localization and translation of target mRNAs. Using a forward genetic approach we identified lethal giant larvae (lgl) locus as a novel interacting gene for dFmr1 in Drosophila. lgl encodes a tumor suppressor, which is required for viability and is thought to function in cellular polarity in association with the cell junctions and the cytoplasmic polarized transport. Through a combined genetic and molecular approach we found that Lgl functions upstream of FMRP in neurons. In addition, Lgl and FMRP form a complex, which contains a set of mRNA and is conserved in mammals. Our central hypothesis is that Lgl controls FMRP/mRNA targeting in neurons and this is required for proper neuronal development.

We have recently obtained evidence that loss of lgl restricts FMRP localization to the cell soma with only a small fraction being present in dendrites in Drosophila cultured neurons. Over-expression of a non- phosphorylatable form of lgl (Lgl3a) in primary neurons affects the polarized distribution of FMRP, with some fraction being misdirected into axons. These data are consistent with a model whereby Lgl acts as a general localization factor for FMRP, while phosphoLgl may function to restrict FMRP distribution to dendrites.

Using recently developed imaging tools to track fluorescently tagged FMRP and associated mRNAs in living neurons we have obtained preliminary evidence that Lgl controls the transport of RNA granules. Current experiments aim to determine what aspects of FMRP/mRNA trafficking are controlled by lgl in neurons. In addition, we have obtained evidence that loss of lgl affects the organization of the Golgi in neurons. Since Lgl and FMRP co-migrate in Golgi associated membrane fractions in the brain, we are currently testing the hypothesis that Lgl may control the localization of FMRP through a mechanism involving the Golgi apparatus. Manipulations of the Golgi using a dominant negative approach support

Exocyst complex in plants

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Exocytosis is the major mechanism for morphogenesis in plant cells; yet we know so little about some aspects of this process. The Rho/Rop GTPase regulatory module is central for initiating exocytotically active domains in plant cell cortex (active cortical domains – ACDs). Most plant cells exhibit several distinct plasma membrane domains, established and maintained by endocytosis-driven membrane constituent recycling. We propose the concept of a “recycling domain” (RD), uniting the ACD and the connected endosomal compartment, as a dynamic spatiotemporal entity. We have recently described the exocyst tethering complex in plant cells. Arabidopsis mutants in exocyst subunits show pleiotropic defects in cell morphogenesis. For example, pollen tube germination and polar growth are seriously compromised, growth of root hairs and stigmatic papilae is impaired, cell elongation in roots and hypocotyls is reduced. Mutant plants are smaller with reduced apical dominance and fewer flower organs, implying dysfunction in the meristem. Co-purification of exocyst subunits in chromatography, and co-localization in growing pollen tube tips, shows that the exocyst is a conserved eukaryotic complex involved in the regulation of cell polarity. Especially due to the multiplicity of its Exo70 subunits, this complex may belong to core regulators of RD organization in plant cells.
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Development of primary mouse hepatocytes as a model system to study polarized endocytic trafficking

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The liver is a multi-functional organ participating in an immense number of physiological processes, e.g. regeneration, toxicology, bile formation, and lipid and glucose metabolism. All those functions are mainly carried out by hepatocytes, which exhibit a very complex and peculiar polarity compared to the so-called “simple polarity” of most epithelia cells. Hepatocytes exist in an arrangement as plates or sheets in the liver, thereby developing several apical and basolateral sides per cell. The apical membrane areas of hepatocytes form a continuous network of bile canaliculi, whereas the basal or sinusoidal domain faces the blood stream, and the lateral sides delineate borders between neighboring hepatocytes. Therefore, specific trafficking routes and retention mechanisms are necessary to ensure proper function of these distinct membrane domains.
The endosomal system plays a key role in maintaining this functional asymmetry or polarity by the formation of spatially distinct intracellular, endocytic networks. Therefore, internalized cargo is delivered specifically via different populations of endosomes to the apical and basolateral membranes ensuring their specificity in trafficking and signaling. Isolated mouse hepatocytes maintain polarity and in vivo functionality when cultured using a 3D collagen sandwich system and provide therefore a good model system to study polarized trafficking. By adapting functional transport assays we can quantitatively measure the flow through the endosomal system using immunofluorescence microscopy.
The aim of our project is to develop a mathematical model that can describe and predict the behavior of the endocytic pathway in hepatocytes with respect to cargo transport as well as signaling. It is clear in fact from resent studies that a complete understanding of the signaling machinery will not be achieved without taken into account the endocytic trafficking of signaling molecules. To pursue this goal, we set out to characterize in a quantitative fashion the endosomal distribution in the apical versus basolateral area by using confocal imaging analysis. For the first time it is possible to visualize the endosomal system in primary hepatocytes at high resolution and answer cell biological questions, like the importance of the endosomal network on the establishment and maintenance of cellular polarity in an in vitro model system of the liver.

Study on the Role of Nap1 in Candida albicans Morphogenesis

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Candida albicans is the most prevalent fungal pathogen in humans, and its ability to switch from yeast to hyphal growth contributes critically to its virulence. Our laboratory has established that the protein kinase Gin4 plays a crucial role in regulating hyphal growth through phosphorylating the septin Cdc11. However, what controls Gin4 remains unclear. Because in Saccharomyces cerevisiae, nucleosome assembly protein 1 (Nap1) is thought to act upstream of Gin4, here I propose to characterize Nap1's role in C. albicans. My preliminary results have shown the following. First, deleting NAP1 causes constitutive cell elongation and invasive growth into agar; second, Nap1 can coimmunoprecitate with Gin4 and septins; and third, the filamentous phenotype of nap1Δ/Δ cells depends on Hgc1, a promoter of hyphal growth. These results appear to suggest that Nap1 acts as a repressor of hyphal growth. Next, I propose to further investigate the functional significance of Nap1's association with Gin4 and septins, and to identify and characterize Nap1's upstream regulators and downstream effectors in order to fully understand Nap1's role in C. albicans hyphal development.

Mechanism of membrane binding by cofilin-1

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The actin cytoskeleton is a vital component of several key cellular and developmental processes in eukaryotes. The structure and dynamics of the actin cytoskeleton are regulated by a wide array of actin-binding proteins, whose activities are controlled by various signal transduction pathways. ADF/cofilins have emerged as key regulators of actin dynamics at the leading edge of motile cells, and these proteins are thus also directly linked to invasion, intravasation, and metastasis of cancer. A number of studies have shown that a membrane phospholipid, PI(4,5)P2, regulates actin polymerization in vitro by binding ADF/cofilins. However, the mechanism of ADF/cofilin - PI(4,5)P2 interaction and its biological relevance are largely unknown. In this study, we elucidate the mechanism of cofilin's interaction with PI(4,5)P2 containing membranes by biophysical and biochemical methods, and identify the PI(4,5)P2-binding sites on cofilin-1 by systematic mutagenesis.

The PDZ protein syntenin controls a molecular network implicated in yolk syncytial layer migration during zebrafish gastrulation.

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PDZ proteins predominate in multicellular organisms and function as molecular scaffolds for signaling complexes. The PDZ protein syntenin interacts with different membrane receptors including syndecan heparan sulphate proteoglycans, and functions as a rate-limiting factor for their plasma membrane recycling in cultured cells. Yet, the relevance of syntenin during development has not been investigated.

Here we show that syntenin is expressed maternally in zebrafish and is essential for early embryogenesis, in particular for the epiboly of the yolk syncytial layer (YSL). Syntenin does not affect actin constriction or microtubule bundling in the YSL but primarily control the autonomous vegetal expansion of this layer. Combination of morpholinos and rescue experiments show that syntenin interaction with phosphatidylinositol 4,5-bisphosphate and ADP-ribosylation factor 6 GTPase, two components supporting its recycling function, is essential for epiboly progression. Finally, we show that syndecans expressed in the YSL, also contributes to syntenin function during epiboly.

In conclusion, our study defines the syntenin pathway as essential for early polarized movement during zebrafish embryogenesis and identifies a first molecular network implicated in YSL migration.

Asymmetry of microtubules remodeling during EGF endocytosis in HeLa cells

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Non-polar cells in dense monolayer are characterized by random positioning of centrosome near nucleus but the cells can acquire asymmetry of centrosome localization under certain conditions. For example, in spare HeLa cell culture during spreading after cytokinesis or in the cells located on the border of small islets the asymmetrical localization of centrosome between nucleus and the leading edge is established. Such localization is supposed to facilitate special role of microtubules (MTs) in mediating vesicular traffic toward and from the plasma membrane domain involved in leading edge formation during cell spreading.
Generally microtubules in leading edge are assumed to retain their radiality. However we have found that interphase microtubules undergo dramatic reorganization in cells being treated with epidermal growth factor, EGF (Kharchenko et al., 2007, Cell Biol Int, 31 (4): 349-359). Importantly, phases of remodeling correlate with stages of EGF-receptor endocytosis: during the first phase (5-20 min) EGF-receptor complexes are localized in early endosomes which are transported along individual MT toward the juxtranuclear region. Later on (up to 60-90 min), during EGF-receptor transition to late endosomes (LE), LE maturation and their interaction with lysosomes, MT system loses its radiality, individual MTs become fragmented and partially depolymerized. The meshwork of fragmented MTs is more obvious in juxtranuclear region near the centriole. Then MT radialilty is re-established. This phenomenon takes place in the cells localized both inside a dense islet and at its border.
Significantly, in the latter case the area of desorganized MTs is localized not around the nucleus but at the side of leading edge while at the opposite pole of the cell MTs remain intact. We have found that the sites of EGF internalization are distributed evenly on the whole surface of the plasma membrane and early endosomes use all cellular MTs equally for transportation to juxtanuclear region. Localization of EGF-containing endosomes becomes asymmetrical upon delivery to the centrosomal area. According to our data signals for MTs reorganization is triggered at some late stage of endosome maturation.We hypothesize that EGF-dependent fragmentation/depolymerisation of MTs could result in activation of some signaling molecules involved in regulation of cell adhesion and/or motility (e.c. PI3-kinase p85) by shedding them from intact MTs during disassembling. In that case such spatial organization of MTs remodeling could provide the signaling essential for leading edge formation.

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