MOLECULAR BASIS FOR MEMBRANE REMODELLING AND ORGANIZATION

Analyses moléculaires de l'organisation et du remodelage des membranes

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Roscoff *(Brittany), France*
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Actine et dynamique membranaire

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La fusion des vésicules

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PROGRAMME

Saturday, Sept. 24

Time	Event
16:00 -- 19:30	Registration
18:15	*Cocktails at CNRS Gulf Stream Hotel*
18:45	*Dinner at CNRS Gulf Stream Hotel*
20:15	Opening remarks
20:30	J.E. Rothman

Sunday, Sept. 25

Time	Event
08:40	P. de Camilli
09:10	E. Boucrot
09:30	S. Suetsugu
10:00	B. Qualmann
10:20	*Coffee break*
10:50	W. Römer
11:20	O. Shupliakov
11:40	N. Sauvonnet
12:00	D. Perrais
13:00	*Lunch at CNRS-Gulf Stream Hotel*
14:30	M. Geyer
15:00	A. Grassart
15:30	G. Scita
16:00	*Coffee break*
16:30	F. Darchen
17:00	S. Groffen
17:20	S. Safieddine
17:50	L. Chernomordik
18:30 20:00	*Dinner at CNRS Gulf Stream Hotel*
20:30 22:30	Poster Session I

Monday, Sept. 26

Time	Event
08:40	T. Rapoport
09:10	B. Antonny
09:40	C. Jackson
10:00	*Coffee break*
10:30	F. Rey
11:00	P. Aspenström
11:30	M. Geli
11:50	R. Beck
13:00	*Lunch at CNRS-Gulf Stream Hotel*
14:30	Excursion or free time
17:30 19:00	Poster session II
19:15 20:30	*Dinner at CNRS Gulf Stream Hotel*
20:40	S. Corey
21:00	S. Meresse
21:30	P. Greer
21:50	M.A. del Pozo

Tuesday, Sept. 27

Time	Event
08:40	P. Lappalainen
09:10	O. Sokolova
09:30	S . Oess
09:50	R. Stanley
10:20	*Coffee break*
10:50	M. Kozlov
11:20	R. Langen
11:50	P. Bassereau
12:10	A. Roux
13:00	*Lunch at CNRS-Gulf Stream Hotel*
14:30	W. Weissenhorn
15:00	J. Zimmerberg
15:30	R. Lundmark
15:50	O. Daumke
16:20	*Coffee break*
16:50	D. Stamou
17:20	B. Goud
17:50	M.-F. Carlier
18:20	Suggestions for next meeting
19:15	*Drinks at CNRS Gulf Stream Hotel*
20:00	*Banquet at CNRS Gulf Stream Hotel*

Wednesday, Sept. 28

Time	Event
08:40	P.-F. Lenne
09:10	C. Valente
09:30	J. Briggs
09:50	H. McMahon
10:20	*Coffee break*
11:00	Bus to Morlaix and Brest

Saturday, September 24
Samedi 24 septembre

CNRS – Hotel Gulf Stream

16:00-20:00	Arrival and Registration *Arrivée et inscription*
18:15-18:45	Welcome drinks *Apéritif d'accueil*
18:45-20:00	Dinner *Dîner*

CNRS – Lecture room

20:15-20:30	Opening remarks **- Barbara WINSOR** *(Strasbourg, France)*
20:30-21:30	***The EMBO Lecture* - (Plenary lecture)** **James E. ROTHMAN** *(New Haven, U.S.A.)* The mechanism of control of neurotransmitter release *Le mécanisme de contrôle de la libération des neurotransmetteurs*

Sunday, September 25
Dimanche 25 septembre

Session I

Membrane curvature in endocytosis and vesicle budding
La courbure membranaire dans l'endocytose et le bourgeonnement des vesicules

Chairperson: Harvey McMahon

08:40-09:10 **Pietro DE CAMILLI** *(New Haven, U.S.A.)*
BAR family proteins in endocytosis
Protéines de la famille BAR dans l'endocytose

09:10-09:30 **Emmanuel BOUCROT** *(Cambridge, United Kingdom)*
Membrane curvature induced by epsin supports clathrin-coated vesicle scission
La courbure membranaire induite par l'epsine favorise la scission des vésicules à manteau de clathrine

09:30-10:00 **Shiro SUETSUGU** *(Tokyo, Japan)*
Phosphorylation of pacsin2 for caveolae endocytosis
La phosphorylation de pacsin2 dans l'endocytose par les caveolae

10:00-10:20 **Brita QUALMANN** *(Jena, Germany)*
Proper synaptic vesicle formation and neuronal network activity critically rely on syndapin I
La Syndapine I est critique pour la formation des vésicules synaptiques et pour l'activité du réseau neuronal

10:20-10:50 ***Coffee break -*** *Pause café*

Session II

Endocytosis and vesicle budding
L'endocytose et le bourgeonnement des vésicules

Chairperson: Harvey McMahon

10:50-11:20 **Winfried RÖMER** *(Freiburg, Germany)*
Synthetic membrane biology: Reconstitution of cellular processes on artificial membrane systems
Biologie synthétique des membranes : Reconstitution de processus cellulaires sur des membranes artificielles

11:20 -11:40 **Oleg SHUPLIAKOV** *(Stockholm, Sweden)*
A pre-fission complex promotes budding of clathrin-coated vesicles during synaptic vesicle recycling
Un complexe de pré-fission favorise le bourgeonnement des vésicules à manteau de clathrine

11:40-12:00 **Nathalie SAUVONNET** *(Paris, France)*

Role of PI 3-kinase in the clathrin-independent endocytosis used by the IL-2 receptors

Rôle de la PI 3-kinase dans l'endocytose clathrine-indépendante des récepteurs IL-2

12:00-12:30 **David PERRAIS** *(Bordeaux, France)*

Assessing the molecular dynamics of clathrin mediated endocytosis using quantitative live-cell imaging

Evaluation de la dynamique moléculaire de l'endocytose clathrine-dépendante par imagerie quantitative dans des cellules vivantes

13:00-14:15 ***Lunch at the Conference Centre (Gulf Stream Hotel)***

Déjeuner au Centre de Conférence (Hôtel Gulf Stream)

Session III

Actin and membrane dynamics

Actine et dynamique membranaire

Chairperson: Sohail Ahmed

14:30-15:00 **Matthias GEYER** *(Dortmund, Germany)*

Assembly of Formin actin nucleators at the plasma membrane

Assemblage des formines, des nucléateurs de l'actine à la membrane plasmique

15:00-15:30 **Alexandre GRASSART** *(Berkeley, U.S.A.)*

Systematic siRNA-based analysis of endocytic protein function by dual-color, real-time imaging in genome-edited cells

Analyse systématique basée sur le siRNA des protéines de l'endocytose par imagerie bi-couleur en temps réél dans les cellules à génome édité

15:30-16:00 **Giorgio SCITA** *(Milan, Italy)*

Membrane and actin dynamic interplay in the plasticity of tumor cell migration

Interactions réciproques dynamiques entre la membrane et l'actine dans la plasticité de la migration des cellules tumorales

16:00-16:30 ***Coffee break -*** *Pause café*

Session IV

Vesicle fusion

La fusion des vésicules

Chairperson: Sohail Ahmed

16:30-17:00 **François DARCHEN** *(Paris, France)*

Membrane tension drives fusion pore dilation during exocytosis

La tension membranaire contrôle la dilatation du pore de fusion au cours de l'exocytose

17:00-17:20 **Alexander GROFFEN** *(Amsterdam, The Netherlands)*
Molecular basis and functional importance of spontaneous neurotransmission
Bases moléculaires et importance fonctionnelle de la neurotransmission spontanée

17:20-17:50 **Saaïd SAFIEDDINE** *(Paris, France)*
Synaptotagmins and otofelin at the auditory hair cell ribbon synapse : Where we are now?
Les synaptotagmines et l'otofeline à la synapse des cellules ciliées du système auditif: Où en sommes nous maintenant ?

17:50-18:20 **Leonid CHERNOMORDIK** *(Bethesda, U.S.A.)*
Fusion in development and in viral entry
La fusion dans le développement et dans l'entrée des virus

18:30-20:00 ***Dinner at the Conference Centre (Gulf Stream Hotel)***
Dîner au Centre de Conférence (Hôtel Gulf Stream)

20:30-21:30 **POSTER SESSION I** *(CNRS-Hotel de France building)*
Communications par affiches – session 1 (Bâtiment CNRS-Hôtel de France)
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21:30-22:30 **POSTER SESSION II** *(CNRS-Hotel de France building)*
Communications par affiches – session I1 (Bâtiment CNRS-Hôtel de France)
See page 79

Monday, September 26
Lundi 26 septembre

Session V

Shaping organelles and organisms
Formation et détection de formes

Chairperson: Jean-François Lenne

08:40-09:10 **Tom RAPOPORT** *(Boston, U.S.A.)*
How the endoplasmic reticulum gets into shape
Comment le réticulum endoplasmique se met en forme

09:10-09:40 **Bruno ANTONNY** *(Valbonne, France)*
ALPS motif, a-synuclein and Spo20p: three amphipathic motifs with contrasting chemistries bind selectively to different types of membranes in vitro and in cells
ALPS, a-synucleine et Spo20p: trois motifs amphipathiques aux chimies contrastées se lient sélectivement à des types de membranes différentes in vitro et dans les cellules

09:40-10:00 **Cathy JACKSON** *(Paris, France)*
Targeting of membrane curvature sensors *in vitro* and in cells is mediated by their dual recognition of membrane shape and lipid composition
Détection de la courbure et la composition des membranes cellulaires par des motifs amphipathiques

10:00-10:30 ***Coffee break - Pause café***

Session VI

From Structure to Function
De la structure à la fonction

Chairperson: Jean-François Lenne

10:30-11:00 **Felix REY** *(Gif-sur-Yvette, France)*
The cell fusion proteins of the “FF” family are homologous to class II viral fusion proteins
Les protéines de fusion cellulaire de la famille «FF» sont homologues aux protéines de fusion virales de la classe II

11:00-11:30 **Pontus ASPENSTRÖM** *(Stockholm, Sweden)*
Rho GTPases in the regulation of membrane trafficking
Les GTPases de la famille Rho dans la régulation du trafic membranaire

11:30-11:50 **María Isabel GELI FERNANDEZ** *(Barcelona, Spain)*
Ultrastructural dynamics of protein complexes involved in endocytic budding
La dynamique ultrastructurale des complexes protéiques du bourgeonnement endocytique

11:50-12:10 **Rainer BECK** *(Heidelberg, Germany)*
Coatomer and dimeric ADP ribosylation factor 1 promote distinct steps in membrane scission
Coatomer et le facteur 1 de la ribosylation de l'ADP dimérique agissent à des étapes distinctes de la scission des membranes

13:00-14:10 ***Lunch at the Conference Centre (Gulf Stream Hotel)***
Déjeuner au Centre de Conférence (Hôtel Gulf Stream)

14:30-17:30 **Free time - Excursion to Batz Island and/or Discussion**
Temps libre - Excursion à l'île de Batz et/ou Discussions

17:30-18:15 **POSTER SESSION III** *(CNRS-Hotel de France building)*
Communications par affiches – session 1 (Bâtiment CNRS-Hôtel de France)
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18:15-19:00 **POSTER SESSION IV** *(CNRS-Hotel de France building)*
Communications par affiches – session I1 (Bâtiment CNRS-Hôtel de France)
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19:15-20:30 ***Dinner at the Conference Centre (Gulf Stream Hotel)***
Dîner au Centre de Conférence (Hôtel Gulf Stream)

Session VII

Membrane dynamics and disease
La dynamique membranaire et les maladies

Chairperson: Violetta Chitu

20:40-21:00 **Seth COREY** *(Chicago, U.S.A.)*
Loss of the Cdc42-interacting protein, CIP4, results in Murine Thrombocytopenia: insights into Wiskott-Aldrich Syndrome
La perte de CIP4, une protéine interagissant avec Cdc42, provoque la thrombocytopénie murine: un nouvel aperçu sur le syndrome de Wiskott-Aldrich

21:00-21:30 **Stéphane MERESSE** *(Marseille, France)*
Manipulation of host cell membranes by *Salmonella* effector proteins
La manipulation des membranes de la cellule hôte par les protéines effectrices de Salmonella

21:30-21:50 **Peter GREER** *(Kingston, Canada)*
The non-receptor tyrosine kinase Fer is required for ErbB2-mediated tumorigenesis
La tyrosine kinase non-recepteur Fer est requis pour la tumorigénèse ErbB2 dépendant

21:50-22:10 **Miguel Angel DEL POZO** *(Madrid, Spain)*
Biomechanical remodeling of the microenvironment by stromal Caveolin-1 favors tumor invasion and metastasis
Le remodelage biomécanique du microenvironnement par la Caveolin-1 stromale favorise l'invasion tumorale et la métastase

Tuesday, September 27
Mardi 27 septembre

Session VIII

Molecular modules that influence membrane curvature I
Modules moléculaires qui affectent la courbure membranaire I

Chairperson: Lennart Brodin

08:40-09:10 **Pekka LAPPALAINEN** *(Helsinki, Finland)*
Regulation of plasma membrane dynamics by I-BAR domain proteins
Régulation de la dynamique de la membrane plasmique par des protéines à domaine I-BAR

09:10-09:30 **Olga SOKOLOVA** *(Moscow, Russia)*
Inactivation of actin-binding protein affects elastic properties of eucaryotic cells
L'inactivation d'une protéine liant l'actine affecte les propriétés élastiques des cellules eucaryotes

09:30-09:50 **Stefanie OESS** *(Francfort, Germany)*
The F-BAR protein NOSTRIN is necessary for proper vascular development in zebrafish and mice
NOSTRIN, une protéine à F-BAR, est nécessaire pour le développement vasculaire chez le poisson zèbre et la souris

09:50-10:20 **Richard STANLEY** *(New York, U.S.A.)*
The F-BAR protein, PSTPIP2, mediates membrane-cytoskeletal interactions in macrophage and osteoclast development and function
La protéine à F-BAR, PSTPIP2, joue un rôle dans les interactions membrane-cytosquelette au cours du développement et dans les fonctions des macrophages et des ostéoclastes

10:20-10:50 ***Coffee break - Pause café***

Session IX

Biophysics of curvature
Biophysique de la courbure

Chairperson: Lennart Brodin

10:50-11:20 **Michael KOZLOV** *(Tel Aviv, Israel)*
Membrane fission can be driven by hydrophobic insertions and controlled by protein scaffolds: a model
La fission membranaire peut être induite par des insertions hydrophobes et régulée par des protéines d'échafaudage: un modèle

11:20-11:50 **Ralf LANGEN** (*Los Angeles, U.S.A.*)
Molecular mechanisms for inducing and sensing membrane curvature
Mécanismes moléculaires de l'induction et de la détection de la courbure membranaire

11:50-12:10 **Patricia BASSEREAU** *(Paris, France)*
Membrane nanotubes: a biophysics tool for understanding the mechanical action of N-BAR proteins on membranes
Les nanotubes de membranes: un outil biophysique pour comprendre l'action mécanique des protéines N-BAR sur les membranes

12:10-12:30 **Aurélien ROUX** *(Geneva, Switzerland)*
Essential elastic and shape parameters govern the dynamics and energetics of dynamin-mediated membrane fission
Des paramètres essentiels d'élasticité et de forme déterminent la dynamique et l'énergétique de la fission membranaire dépendante de la dynamine

13:00-14:15 ***Lunch at the Conference Centre (Gulf Stream Hotel)***
Déjeuner au Centre de Conférence (Hôtel Gulf Stream)

Session X

Molecular modules that influence membrane curvature II

Modules moléculaires qui affectent la courbure membranaire II

Chairperson: Pietro De Camilli

14:30-15:00 **Winfried WEISSENHORN** *(Grenoble, France)*
ESCRT-III regulation, assembly and membrane remodeling
La régulation, l'assemblage et le remodelage membranaire de ESCRT-III

15:00-15:30 **Joshua ZIMMERBERG (***Bethesda, U.S.A.)*
The organization and remodeling of biological membrane
L'organisation et remodelage des membranes biologiques

15:30-15:50 **Richard LUNDMARK** *(Umeå, Sweden)*
EHD2 remodels caveolae membranes via specific ATP-driven targeting and oligomerisation
EHD2 remodèle les membranes des caveolae par un ciblage spécifique dépendant de l'ATP et par oligomérisation

15:50-16:20 **Oliver DAUMKE** *(Berlin, Germany)*
Structure, oligomerization and mechanism of dynamin superfamily proteins
Structure, oligomérisation et mécanisme d'action des protéines de la superfamille des dynamines

16:20-16:50 ***Coffee break - Pause café***

Session XI

Methods applied to membrane curvature, insertion and diffusion

Méthodes appliquées à la courbure des membranes, à l'insertion et à la diffusion

Chairperson: Pietro De Camilli

16:50-17:20 **Dimitrios STAMOU** *(Copenhagen, Denmark)*
Three distinct pathways by which curvature modulates the structure and function of membranes
Trois voies distinctes par lesquelles la courbure régule la structure et la fonction des membranes

17:20-17:50 **Bruno GOUD** *(Paris, France)*
Rab GTPases and actin-based motors
Rab GTPases et les moteurs associés à l'actine

17:50-18:20 **Marie-France CARLIER** *(Gif-sur-Yvette, France)*
Coupled dynamics of actin filaments and membranes in motile processes: a reconstitution approach
La dynamique couplée des filaments d'actine et des membranes dans des processus motiles : une approche par la reconstitution

18:20-18:50 Suggestions for next meeting; election of next chair and vice-chair: fill out evaluation forms
Suggestions pour la prochaine rencontre; élection des prochains Chair et Vice-Chair ; remplissage des formulaires d'évaluation

19:15-20:00 ***Drinks - Apéritif***

20:00-22:00 ***Banquet at the Conference Centre (Gulf Stream Hotel)***
Banquet au Centre de Conférence (Hôtel Gulf Stream)

Wednesday, September 28
Mercredi 28 septembre

Session XII

Membrane curvature and fission
La courbure des membranes et la fission

Chairperson: Leon Chernomordik

08:40-09:10 **Pierre-François LENNE** *(Marseille, France)*
Cortical forces in cell shape changes and tissue morphogenesis
Les forces corticales dans les changements de forme cellulaire et dans la morphogenèse des tissus

09:10-09:30 **Carmen VALENTE** *(Naples, Italy)*
14-3-3γ regulates CtBP1-S/BARS-mediated fission of post-Golgi carriers
14-3-3γ régule la fission CtBP1-S/BARS-dépendante des transporteurs post-Golgiens

09:30-09:50 **John BRIGGS** *(Heidelberg, Germany)*
Visualizing vesicle and virus budding by electron microscopy
Visualisation du bourgeonnement des vésicules et des virus par microscopie électronique

09:50-10:20 **Harvey McMAHON** *(Cambridge, United Kingdom)*
Molecular determinants of membrane fusion and fission
Déterminants moléculaires de la fusion et de la fission des membranes

10:20-10:45 **Coffee break -** ***Pause café***

Bus to Morlaix railway station

11:00 ➔ Departure from CNRS-Hotel de France / Ibis hotel
11:10 ➔ Departure from CNRS-Hotel Gulf Stream

Bus to Brest airport

11:00 ➔ Departure from CNRS-Hotel de France / Ibis hotel
11:10 ➔ Departure from CNRS-Hotel Gulf Stream

13:15 ➔ Departure from CNRS-Hotel de France / Ibis hotel
13:25 ➔ Departure from CNRS-Hotel Gulf Stream

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The Mechanism of Control of Neurotransmitter Release

James Rothman
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SNAREs fuse cellular membranes, including synaptic vesicles with pre-synaptic plasma membranes to enable synaptic transmission. SNAREs fuse membranes automatically, so a clamp is needed to prevent premature transmitter release, provided by the protein complexin. Recent studies have established the structural and biochemical mechanism of clamping by complexin involving a co-operative array of partly zippered SNAREpins whose disolution can explain the syncronicity of transmitter release when the calcium sensor, synaptotagmin, perturbs the array.

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BAR family proteins in endocytosis

Pietro De Camilli
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Proteins containing modules of the BAR superfamily play an important role in coordinating bilayer deformations implicit in the formation of endocytic vesicles with other changes that must occur at endocytic sites for the progression of the endocytic reaction. The curved BAR domain dimer helps generate, propagate and stabilize curvature, often in coordination with flanking membrane binding amino acid regions. Additional domains present in these proteins control, via indirect or indirect interactions, the recruitment of other endocytc factors, the local actin cytoskeleton, or the chemistry of the bilayer. My talk will focus on the role of two proteins of the BAR superfamily. The first is endophilin, an N-BAR containing protein that plays an important function in endocytic recycling at neuronal synapses via its recruitment of the PI(4,5)P2 phospahatase synaptojanin. The second is FBP17, which contains an F-BAR module and belongs to a family of proteins (FBP17/CIP4/Toca1 family) that have been characterized as actin regulatory factors in contexts unrelated to endocytosis. Our work suggests that FBP17 is a component of a protein network that couples membrane bending underlying endocytosis to Cdc42-dependent actin nucleation.

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Membrane curvature induced by epsin supports clathrin-coated vesicle scission

Boucrot E.[1], Camdere G.[1], Pick A.[2], Evergren E.[1], Liska N.[1], Koslov M.M.[2] and McMahon H.T.[1]
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Membrane fission and membrane fusion involve rearrangements of membrane continuity requiring action of specialized protein modules. With Misha Kozlov we suggest a mechanistic and quantitative basis for membrane fission by shallow hydrophobic insertions. We demonstrate the biological feasibility of this mechanism with epsin ENTH domains inserting their N-terminal helices into the membrane matrix. With a new assay to quantitatively measure vesiculation in a reconstituted system, we show that epsin ENTH domain is sufficient to induce membrane fission. In vivo, we found that epsin proteins are required for fission of clathrin-coated pits from the plasma membrane. Knocking-down the levels of epsin proteins was sufficient to block maturation of clathrin-coated pits. Importantly, we found that manipulating the levels of epsin proteins in cells rescue the block of clathrin-coated vesicle fission in cells where the mechanoenzyme, dynamin, has been depleted.
Furthermore, we show how BAR-domain type scaffolds (taken from endophilin, amphiphysin and GRAF) put limits on the extent of membrane fission driven by the hydrophobic insertions. We observe that N-BAR domains with progressively more amphipathic helices give more membrane fission. Conversely, adding amphipathic helices to the BAR domain of centaurin triggers this domain to support membrane fission both *in vitro* and *in vivo*.
This mechanism of fission can likely be applied to many membrane remodelling events in the cell and opens the possibility of membrane fission in the absence of known mechanoenzymes such as dynamin.

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Phosphorylation of pacsin2 for caveolae endocytosis

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The F-BAR domain protein, protein kinase C and casein kinase substrate in neurons 2 (PACSIN2) / synaptic, dynamin-associated protein (syndapin II) is involved in caveolae formation and endocytosis. However, how caveolae is regulated by PACSIN2 is unknown. We found that PACSIN2 was phosphorylated by PKC in vitro and in cells. Membrane tubulation ability of PACSIN2 was not perturbed significantly by the phosphorylation in vitro, however, the tubulation induced by PACSIN2 in cells was significantly reduced by PKC activation. This inhibition of tubulation by PKC was suppressed by the expression of dominant negative dynamin 2. Furthermore, activation of PKC changed the association of dynamin 2 to PACSIN2. These results indicated that dynamin assembly by PACSIN2 was regulated by PKC, and the activation of PKC could regulate caveolae internalization.

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Proper synaptic vesicle formation and neuronal network activity critically rely on syndapin I

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Synaptic transmission relies on effective and accurate compensatory endocytosis. F-BAR proteins are predestined to serve as membrane curvature sensors and/or inducers and may thereby support membrane remodeling processes, yet, their *in vivo* functions urgently await disclosure. We demonstrate that the F-BAR protein syndapin I is crucial for proper brain function. Syndapin I knock-out mice suffer from seizures, a phenotype consistent with excessive hippocampal network activity. Loss of syndapin I causes defects in presynaptic membrane trafficking processes evident by loss of synaptic vesicle size control and defects in synaptic activity. Upon high-capacity retrieval accumulation of endocytic intermediates are observed in the ribbon synapses of the retina. Detailed molecular analyses demonstrate that syndapin I plays an important role in the recruitment of all dynamin isoforms, central players in vesicle fission reactions, to the membrane. Consistently, syndapin I KO mice share phenotypes with dynamin I KO mice, whereas their seizure phenotype is very reminiscent of *fitful* mice expressing a mutant dynamin. Syndapin I thus acts as pivotal membrane anchoring factor for dynamins during regeneration of synaptic vesicles.

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Synthetic membrane biology: Reconstitution of cellular processes on artificial membrane systems

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Specific interactions of lectins with glycosphingolipids lead to tubular plasma membrane invaginations and cellular uptake of pathogens and pathogenic products Each cell produces a distinct array of cell surface glycans that carries rich structural information unique to that cell. It is not surprising that multiple carbohydrate-binding proteins (“lectins”) have evolved to use glycans as receptors to mediate cell surface biology. Different pathogens

or pathogenic factors, like simian virus 40 and polyoma virus, cholera and Shiga toxins, also exploit host cell plasma membrane glycans to enter and infect cells. After binding to the glycosphingolipids GM1, GD1a and Gb3, respectively, these molecules enter cells via endocytic routes that do not involve the elaborate network of clathrin.
These bacterial toxins and animal viruses can induce tubular plasma membrane invaginations without the help of the cytosolic protein machinery through the dynamic construction of protein-lipid nanodomains whose intrinsic properties lead to membrane bending and invagination. Depletion of cellular energy or direct inhibition of dynamin or actin function on cells allow to uncouple membrane invagination from the subsequent scission step suggesting that cellular factors are required not for the formation of the invaginations, but for their processing. The formation of deep membrane invaginations and tubules occurs not only in the plasma membrane of cells, but can also be reconstituted in giant unilamellar vesicles. These studies have uncovered a previously unknown mechanism for generating negative membrane curvature, and they have created a new paradigm that allows conceptualizing why endocytic coats are not detected at many sites of clathrin-independent endocytosis. Lectin-induced tubular membrane invaginations are poised in a membrane environment whose lipid and protein composition supports domain formation. The scission of these plasma membrane invaginations is preceded by cholesterol-dependent membrane reorganization, and correlates with the formation of membrane domains on model membranes, suggesting that domain boundary forces are driving tubule membrane constriction. Actin triggers scission by inducing such membrane reorganization processes that may function independently from or in synergy with pinchase activity.

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A pre-fission complex promotes budding of clathrin-coated vesicles during synaptic vesicle recycling

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The high rate of clathrin-mediated vesicle recycling in synapses is maintained by an unique set of endocytic proteins and interactions. Recycling of synaptic vesicles after neurotransmitter release is crucial for sustained transmission. This membrane retrieval process occurs mainly through the clathrin-mediated endocytosis pathway. Dynamin, a large GTPase, is believed to mediate the fission of invaginated clathrin-coated pits (ccps) from the plasma membrane, resulting in clathrin-coated vesicles. We found that the endocytic protein endophilin co-localizes with dynamin at a restricted part of the neck of the clathrin-coated pit in intact lamprey giant synapses. Blocking endophilin-SH3 domain interactions reduces dynamin accumulation at the neck and prevents formation of elongated necks that are normally observed in the presence of GTPγS. Protein-lipid tubules formed with endophilin 1 and dynamin 1 together, and visualized by cryo-electron microscopy, are thinner than those formed by dynamin alone. Tubulation efficiency and the amount of dynamin recruited to lipid tubes are dramatically increased in the presence of endophilin. Our results indicate that endophilin and dynamin form a "pre-fission complex" at the bottom of the coat of ccps and suggest a mechanism which accelerates dynamin-mediated budding of newly formed synaptic vesicles.

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Role of PI 3-kinase in the clathrin-independent endocytosis used by the IL-2 receptors

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Receptor-mediated endocytosis is a basic and essential process used by eukaryotic cells to internalize, actively and specifically, a wide range of molecules. These molecules include nutrient, growth factors, toxins but also virus or bacteria. Several endocytic routes exist, dependent on or independent of clathrin. This study focuses on a poorly characterized mechanism, the clathrin-independent endocytosis, used by several cytokine receptors such as the interleukin 2 receptors (IL-2R). So far few factors have been implicated in this mechanism. Some, like dynamin, actin and cortactin, are common actors of various endocytic processes. Others are specifically required in this pathway, in contrast to the clathrindependent one. This is the case of Rac1 and its downstream targets the p21-activated kinases (Paks) that were shown to regulate the function of cortactin. Interestingly, the p85α regulatory subunit of phosphatidylinositol 3-kinase (PI 3-Kinase) was shown to bind Rac1. In an attempt to identify the upstream activator of Rac1 in IL-2R entry, we tested the role of p85α and PI 3- Kinase. We showed

that the overexpression of p85α mutants, the depletion of p85α, or the use of PI 3-Kinase inhibitors, led to the specific inhibition of IL-2R endocytosis, in contrast to transferrin uptake. Furthermore, we found that the inhibitory effect of p85α mutant can be rescued by the overexpression of either Rac1 or the active form of Pak1. In addition, we observed that IL-2R and p85α are partially co-localized at the plasma membrane and they can be co-immunoprecipitated. These data strongly suggest an interaction between IL-2R and p85α. Therefore, we propose a model where the association of PI 3-Kinase with IL-2R, via the p85α regulatory subunit, could lead to the recruitment and/or the activation of Rac1 in the clathrin- and caveolin-independent entry used by IL-2R uptake.

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Assessing the molecular dynamics of clathrin mediated endocytosis using quantitative live-cell imaging

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Clathrin-mediated endocytosis (CME) is a fundamental process of all eukaryotic cells which is controlled by a large number proteins and lipids. In the time needed (~1 min) for the formation of clathrin coated vesicles (CCVs), many proteins act successively and in parallel to ensure the concentration of receptor cargo, invagination of the plasma membrane, scission, movement and uncoating of the vesicle. I will present an assay designed to detect the formation of endocytic vesicles containing transferrin receptor with a time resolution of 2 seconds (Merrifield et al. 2005). This assay uses Total Internal Reflection Fluorescence (TIRF) microscopy and alternating pH changes to distinguish newly formed vesicles from clusters of receptors still on the plasma membrane. In various cell types, including NIH 3T3 cells, HeLa cells and cultured hippocampal neurones, we can detect many vesicles and average several hundred 'endocytic events' to construct recruitment profiles of associated proteins fused with mCherry. With this methodology, I will present results on the dynamic molecular organization of CME in 3T3 cells (Taylor et al. 2011) and the cellular organization of exocytic and endocytic sites in cultured hippocampal neurones.

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Assembly of Formin actin nucleators at the plasma membrane

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Regulation of the actin cytoskeleton is a key process for the stability and motility of eukaryotic cells. Besides Arp2/3 and WH2 domain containing proteins a diverse family of formin proteins has recently been recognized as actin nucleators and potent polymerization factors of actin filaments. Actin filaments can be further arranged in a broad variety of higher order structures, such as filopodia, lamellipodia or actin stress fibers; their assembly and association with motor proteins can generate forces, which mediate cellular processes such as migration, cell division, endocytosis, exocytosis, organelle morphology, transport during gene transcription and muscle contraction. The human formin FMNL2 is myristoylated and associates to the plasma membrane upon activation by the GTPase Cdc42. Besides the N-terminal lipidation signal, we have identified a basic surface patch on the armadillo repeat structure of the N-terminal regulation domain in FMNL2 that contributes to membrane binding. Unexpectedly, 12 arginine or lysine residues from different repeats assembled to one cluster that strongly supports membrane association of the FMNL2 protein. In addition, dimerization of the regulation domains by a C-terminal coiled coil segment led to a stable domain assembly. Together with the N-terminal myristoylation signal and the lipidation motifs of Cdc42 the dimer assembly results in the formation of a convex protein surface. Such assembly might support formation of negatively curved surface patches at the plasma membrane that could act as seeds for filopodia outgrowth. In a second part of my talk, I will report on fluorescence spectroscopy techniques that allow the kinetic analysis and biochemical characterization of membrane binding

with respect to membrane curvature. The contribution of an amphipathic helix to the sensing and shaping of positive membrane curvature is exemplarily shown for the myristoylated HIV-1 Nef protein. These techniques provide a quantitative measure for the analysis of curvature-dependent protein binding.

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Systematic siRNA-based analysis of endocytic protein function by dual-color, real-time imaging in genome-edited cells

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By combining siRNAs with dual-color-TIRF imaging and multi-parametric quantitative analysis of genome-edited mammalian cells, we detected distinct functions for endocytic proteins at the plasma membrane. Clathrin-mediated endocytosis (CME) is the best-studied endocytic pathway. Numerous molecular actors have been identified, and live-cell imaging studies have revealed the sequence and timing of their recruitment during cargo uptake. Recent application of high precision microscopy and quantitative analysis to studies of clathrin-coated structure (CCS) dynamics during endocytosis revealed high heterogeneity. The origin of this heterogeneity remains unclear, but recent studies suggest that use of ectopically overexpressed fluorescently tagged endocytic proteins may contribute. While discovering the nature of the heterogeneity may provide insights into endocytic mechanisms, the heterogeneity also complicates analysis of endocytic protein functions. To overcome problems caused by protein overexpression, our laboratory has used Zinc Finger Nuclease (ZFN) technology to express RFP- and GFP-tagged versions of clathrin and dynamin, respectively, preserving their endogenous levels and stoichiometry. Interestingly, this genome-edited cell line displays a more robust and regular CME process than was previously observed in cells overexpressing endocytic proteins. When we performed an siRNA analysis of endocytic protein functions in this cell line, we were able to sensitively detect loss of function phenotypes. Based on this analysis we have grouped endocytic proteins into distinct functional classes.

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Membrane and actin dynamic interplay in the plasticity of tumor cell migration

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Cells, and in particular tumor cells, can adopt different modes of cell motility. The ability to switch between diverse modes of migration enables tumors to adapt to micro-environmental conditions and to metastasize. The critical pathways and cellular processes underlying the plasticity of tumor cell motility have only begun to be identified. An appealing hypothesis, supported by recent evidence, is that endocytosis, originally thought of as a device to internalize nutrients and membrane-bound molecules, is a connectivity infrastructure (which we call “the EndocyticMatrix”) necessary for the execution of various cellular programs. A primary role of the Endocytic Matrix is the delivery of space- and time-resolved signals to the cell, and is thus essential for the execution of polarized functions during 3D cell migration and invasion. Here, we will focus on the endocytic and signaling functions of Rab5, a smallGTPases essential for endosome biogenesis. We will discuss experimental evidence that support the general paradigm that intracellular trafficking, controlled by Rab5, is needed to re-direct molecules to restricted regions of the plasma membrane and to couple the formation of Rac-dependent migratory protrusions with proteolytically active adhesive site, ultimately mediating tumor cell invasion in 3D matrices.

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Membrane tension drives fusion pore dilation during exocytosis

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Membrane fusion underlies several physiological processes including exocytosis of hormones and neurotransmitters. Fusion pore dynamics controls the release of vesicle content but is poorly understood. We found that the small GTPase Cdc42 controls the dilation of the fusion pore. Cdc42 had no effect on the probability of vesicle exocytosis silencing in neuroendocrine cells but compromised the enlargement of the fusion pore, as measured by carbon fiber amperometry and evanescent wave microscopy. As a consequence, the mode of vesicle exocytosis was shifted from full-collapse fusion to kiss-and-run in Cdc42 knockdown cells. Remarkably, using the tether pulling technique, we found that Cdc42 silencing reduced the membrane tension. Moreover, the artificial increase of membrane tension restored fusion pore enlargement in these cells. We conclude that membrane tension is a driving force for fusion pore dilation and that Cdc42 is a key regulator of this force.

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Molecular basis and functional importance of spontaneous neurotransmission:

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It has recently become clear that neurotransmitter secretion can be triggered by various Ca^{2+} sensing proteins including synaptotagmins, doc2 proteins and ferlins. Alterations in the balance between fast and 'spontaneous' release after genetic deletion of specific Ca^{2+} sensors suggest that alternative sensors may compete for components of the secretory apparatus. In biochemical experiments they indeed compete for SNARE binding. Here we analyzed mouse neurons lacking Doc2A, Doc2B and Synaptotagmin-1 expressing various Ca^{2+} sensor constructs. These manipulations strongly affected the balance between different release components, consistent with the competition hypothesis and indicating that additional Ca^{2+} sensors remain to be identified. To reveal the physiological importance of spontaneous release in mice we exploited the four-fold reduction in spontaneous release frequency in Doc2b-deficient Purkinje-Purkinje synapses of the cerebellum[1]. Results from behavioural experiments indicate that spontaneous inhibitory events in these synapses contribute importantly to motor performance in young mice, whereas this system does not appear to be essential during adulthood.

[1] Groffen AJ, et al., Doc2b is a high-affinity Ca2+ sensor for spontaneous neurotransmitter release. Science. 2010 Mar 26;327(5973):1614-8.

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Synaptotagmins and otofelin at the auditory hair cell ribbon synapse: Where we are now?

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The sense of hearing rely primarily on the sensory cell of the cochlea, inner and outer hair cells. The synapses between inner hair cells (IHCs) and the primary auditory neurons are the first chemical synapses to encode sound waves into electrical signals, by releasing glutamate thereby exciting the postsynaptic afferent fibers. The transduction of the sound stimulus into an electrical signal occurs when the IHC depolarizes, resulting in an increase of Ca2+ influx raising the calcium concentration at the active zone. The Ca2+ binds to its sensor thereby triggering synaptic vesicles fusion with the presynaptic plasma membrane. The IHC ribbon synapse uses a SNARE (soluble N-ethylmaleimide-sensitive factor (NSF) attachment receptor) complex similar to that of the

CNS synapses. Curiously, the Syt1 and Syt2 proteins, considered as the major Ca^{2+} sensors at the CNS synapses, were not detected in mature IHCs, suggesting that the IHC synapses use an yet unidentified "synaptotagmin-like" calcium sensor. Genetic screening for a hereditary form of human deafness have led to the identification of otoferlin, a six C2 domain protein, which is defective in the recessive form of human deafness DFNB9. The study of the corresponding null mutant mice led us to suggest otoferlin as the major Ca2+ sensor at the IHC ribbon synapse. In spite of significant data accumulated during the past five years supporting the role of the otoferlin like Ca^{2+} sensor at the IHC ribbon synapse, this assumption, however, has been recenntly challenged. My presntation will highlight and disccuss the new findings regrading the current concepts about otoferlin and synaptotamins function at mature IHC ribbon synapse.

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Fusion in development and in viral entry

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In our recent work on mechanisms of viral and developmental membrane fusion, we have focused on dengue virus, an important human pathogen that infects up to 100 million people annually world-wide, including cases in US and in France, and on cell-to-cell fusion that generates our skeletal muscles - one third of human weight. Dengue virus (DENV) introduces its RNA into cells by fusion between viral envelope and endosomal membrane. Activation of DENV protein fusogen glycoprotein E is triggered already at pH characteristic for early endosomes. However most of the virions have been reported to fuse only in late endosomes. We hypothesized that this finding as well as a very low efficiency of dengue virus fusion to plasma membrane of mammalian cells can be explained by lipid dependence of dengue virus fusion machinery. Indeed we found that effective virus fusion to plasma- and intracellular- membranes, as well as to protein-free liposomes, requires the target membrane to contain anionic lipids such as bis(monoacylglycero)phosphate, a lipid specific to late endosomes. Anionic lipids act downstream of low-pH-dependent fusion stages and promote the advance from the earliest hemifusion intermediates to the fusion pore opening. To reach anionic lipid-enriched late endosomes, DENV travels through acidified early endosomes but we found that low pH-dependent loss of fusogenic properties of the virions is relatively slow in the presence of anionic lipid-free target membranes. We propose that anionic lipid-dependence of DENV fusion machinery protects it against premature irreversible restructuring and inactivation and ensures viral fusion in late endosomes, where the virus encounters anionic lipids for the first time during entry. Better understanding of the mechanisms of DENV fusion and two novel quantitative high-throughput assays for measuring virus fusion will hopefully facilitate development of antivirals including those targeting interactions between dengue E protein and anionic lipids.

Cell-to-cell fusion is a key stage in many developmental processes including fertilization and formation of bone, placenta, and muscles, as well as in mature organisms in muscle repair and in formation of multinucleated giant cells during inflammatory reactions. A major challenge in studying molecular mechanisms of membrane fusion is to isolate the actual fusion stage from the preceding stages that prime the cells to fusion. In one of the better characterized examples of developmental cell fusion, fusion between myoblasts in formation of muscle fibers a number of key molecular players involved in myoblast differentiation, the acquirement of fusion competence, the recognition and adhesion between myoblasts have been identified. However, we still do not know the specific proteins that fuse lipid bilayers of two plasma membranes into one. We isolated fusion stage in myotube formation by murine myoblasts (C2C12 cells) labeled with different membrane and cytosolic probes by blocking myotube formation immediately prior to fusion with reversible hemifusion-inhibitor lysophosphatidylcholine. This approach accumulates cells at a ready-to-fuse stage and, thus, synchronizes fusion upon lifting the inhibitor. Isolation of the fusion stage allowed us to explore myoblast fusion pathway and identify annexins A1 and A5 as proteins that are present at cell surface at the time of fusion and play an important role in fusion. To explore whether annexins are capable of mediating fusion in the absence of other proteins we used phosphatidylserine-containing liposomes. Both A1 and A5 were found to induce lipid mixing between these liposomes. Annexins A1 and A5 drive fusion beyond early hemifusion stage, as evidenced by lipid mixing between the inner leaflets of liposomal membranes. The annexin-mediated fusion depends on the presence of calcium, anionic lipids and insertion of amphiphilic regions of annexins into target membranes. Our results support the hypothesis that annexins directly mediate myoblast fusion by mechanisms similar to those discussed for viral and intracellular protein fusogens rather than regulate cell fusion via other proteins.

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How the endoplasmic reticulum gets into shape

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How is the characteristic shape of a membrane-bound organelle achieved? We have addressed the mechanism by which the morphology of the endoplasmic reticulum (ER) is generated. ER tubules are shaped by two families of integral membrane proteins, the reticulons and DP1/Yop1p, which are necessary and sufficient for tubule formation. These proteins may use hydrophobic insertion and scaffolding mechanisms to shape lipid bilayers into tubules. The interconnection of ER tubules in mammalian cells requires the atlastins, membrane-bound GTPases of the dynamin family, which probably promote homotypic fusion of the tubules. A similar GTPase in yeast, Sey1p, may have an analogous function. Our recent results suggest that the reticulons and DP1/Yop1p are also a major determinant of peripheral ER sheets, which can be further stabilized by sheet-promoting proteins.

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ALPS motif, a-synuclein and Spo20p: three amphipathic motifs with contrasting chemistries bind selectively to different types of membranes in vitro and in cells

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Many cytosolic proteins contain amphipathic helical motifs that serve as reversible membrane anchors. The chemistry of these membrane curvature sensors is sometimes remarkably different. This suggests that, in addition to the segregation between hydrophobic and polar residues, other parameters can tune membrane adsorption. We have compared 3 amphipathic motifs with contrasting chemistries for their ability to adsorb to lipid membranes in vivo and in vitro: the ALPS motif, which has a well-developed hydrophobic face but few charged residues on its polar face; a-synuclein, which has a poor hydrophobic face and many charged residues in its polar face; Spo20p, which has a basic polar face and a classical hydrophobic face. We show that the unbalanced chemistry of both ALPS motifs and a-synuclein is critical for their ability to sense membrane curvature. However only a-synuclein requires negatively charged lipids for binding. When expressed in yeast cells, these two curvature sensors were targeted to different classes of vesicles, those of the early secretory pathway for ALPS motifs and to negatively charged endocytic/post-Golgi vesicles in the case of a-synuclein. These findings show that complementary curvature sensing mechanisms operate in the two major lipid environments of the cell, and that curvature sensors can discriminate between vesicles of different lipid composition. In contrast to the ALPS motif and to a-synuclein, the Spo20 helix is not a sensor of membrane curvature but rather a sensor of charge density, integrating the contribution of all anionic lipids (PS, PIP_2 and PA) present in the membrane.

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Targeting of membrane curvature sensors *in vitro* and in cells is mediated by their dual recognition of membrane shape and lipid composition

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Membrane curvature sensors have different structures and chemistries. ALPS motifs bind specifically to highly curved membranes due to the fact that they are unbalanced lipid-binding amphipathic helices, having a well-developed hydrophobic face but few charged residues on their polar face. Numerous ALPS motifs are found in proteins that function in the early secretory pathway. A second type of amphipathic helical curvature sensor is

exemplified by α-synuclein, a causative agent of Parkinson's disease that is localized to synaptic vesicles. α-synuclein is also an unbalanced amphipathic helix, but opposite to ALPS motifs, has a well developed polar face and a poor hydrophobic face. Hence both ALPS motifs and α-synuclein have a "weakness" that makes positive membrane curvature mandatory for their binding. However, these curvature sensors have opposite chemistries, and direct *in vitro* comparisons of ALPS motifs and α-synuclein demonstrate that they have different lipid requirements for binding, preferring lipid compositions complementary to each one's distinct chemical properties. When expressed in yeast cells, these two curvature sensors were targeted to different classes of vesicles, those of the early secretory pathway for ALPS motifs and to negatively charged endocytic/post-Golgi vesicles in the case of α-synuclein. The lipid composition of the vesicles that α-synuclein and ALPS motifs associate with in cells correlate well with their *in vitro* lipid binding properties. These findings show that complementary curvature sensing mechanisms operate in the two major lipid environments of the cell, and that curvature sensors can discriminate between vesicles of different lipid composition.

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The cell fusion proteins of the "FF" family are homologous to class II viral fusion proteins

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Structural studies of viral membrane fusion proteins have provided key information to understand the process of virus-cell membrane fusion, which is important during virus entry. Similar principles were inferred from structural studies of SNARE proteins, responsible for fusion of intracellular vesicles with their target membranes within the cell. No data are currently available on cellular fusion proteins responsible for cell-cell fusion, a process that is of high relevance during developmental biology and organogenesis.

We will describe the crystal structure of a cell fusion protein from the nematode *C. elegans*, termed "Eff-1", where it plays a key role during organogenesis. This protein is the prototype of the "Fusion Family" (FF) of proteins recently identified. The structure provides unanticipated evolutionary links with the class II viral fusion proteins observed in regular enveloped viruses. Eff-1 folds as three beta-sheet-rich domains domains, assembled as a trimer organized as in the post-fusion structures of the alphaviruses and flaviviruses envelope proteins. This type of fusion proteins had so far been observed only in regular envelope viruses, where they make an icosahedral coat completely enclosing the viral membrane in their pre-fusion form. In the presentation, we will describe the structure and suggest a model for its putative mechanism of action.

Keywords: cell-cell fusion, developmental biology, structural biology, class II viral fusion proteins

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Rho GTPases in the regulation of membrane trafficking

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The Rho GTPases have important roles in the regulation of the actin filament system. Thereby, they are essential for vital cellular functions, such as cell migration, cell contraction and cell division. The best studied Rho family members RhoA, Rac1 and Cdc42 have been linked to the pathways involved in regulating actin dynamics by binding to actin nucleation promoting factors (NPFs), such as WASP/WAVE and Diaphanous-related formins. Additionally, Rho GTPases participate in the regulation of endocytosis and vesicle trafficking, however the molecular mechanisms underlying this control remain elusive. The F-BAR proteins, which have the capacity to bind and deform lipid membranes, were originally identified as binding partners for Rho GTPases. Thus, the F-BAR proteins serve to coordinate cytoskeletal reconstructions and lipid membrane dynamics.

We have studied the role of some F-BAR proteins in signalling downstream of Rho GTPases. We found that the three CIP4-like proteins vary in their binding specificity for Rho GTPases, as well as in their abilities to catalyse membrane tubulation and actin reorganization. In addition, we show that the less studied Rho members, e.g. RhoD and Rif, coordinate membrane trafficking and actin dynamics, however they act through other types of target proteins. We will discuss examples of how distinct Rho family effectors collaborate to regulate important biological processes, such as cytoskeletal integrity, transmembrane receptor trafficking and Golgi homeostasis.

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Ultrastructural dynamics of protein complexes involved in endocytic budding

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Our group has developed a methodology to statistically analyze the distribution of colloidal gold particles immunodecorating proteins associated to endocytic invaginations in *S. cerevisiae* (Idrissi *et al.* 2008 J. Cell Biol). Using this methodology, we have been able to describe the localization of nine proteins involved in endocytic budding along plasma membrane invaginations, with a resolution of 8 nm. The data has demonstrated that the primary endocytic profiles in yeast are tubular structures of 50 nm in diameter and up to 180 nm in length, which carry an hemispherical clathrin coat, which occupies 30 to 40 nm of the tip (Idrissi *et al.* 2008 J. Cell Biol). The statistical processing of the data has also established that the length of the invagination can be used as a parameter to describe their age (Idrissi *et al.* 2008 J. Cell Biol). We have now extended this kind of analysis to a total of twenty proteins, which include the clathrin heavy chain (Chc1), the epsins Ent1 and Ent2, the endocytic adaptor Sla1, the Hip1R homologue Sla2, the eps15 homologues Pan1 and Ede1, the BAR-containing proteins Syp1/FCHO1, Bzz1/Nwk1 and Rvs167/Endophilin, actin (Act1) and the actin binding proteins Abp1, Sac6/Fimbrin and Cap1/Capping protein, the Arp2/3 complex and their activators Las17/N-WASP, Vrp1/WIP and Myo5/myosin-I, the Las17/WASP inhibitor Bcc1 and the synaptojanin Slj2. In addition, we have investigated the morphology of the invaginations after interfering with the function of key components of the endocytic machinery. The analysis of the data provides a comprehensive map of the distribution of protein complexes along endocytic invaginations at an intermediate resolution between the live-cell fluorescence microscopy and the X-Ray crystallography and unveils unexpected changes in the localization of certain proteins as the profile matures. In addition, the results provide strong evidence supporting that endocytic budding in yeast can be dissected in at least 4 stages supported by distinct molecular mechanisms and exhibiting different kinetics.

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Coatomer and dimeric ADP ribosylation factor 1 promote distinct steps in membrane scission

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Formation of coated vesicles requires two striking manipulations of the lipid bilayer. First, membrane curvature is induced to drive bud formation. Second, a scission reaction at the bud neck releases the vesicle. Using a reconstituted system for COPI vesicle formation from purified components we find that a dimerization-deficient Arf1 mutant, which does not display the ability to modulate membrane curvature in vitro or to drive formation of coated vesicles, is able to recruit coatomer to allow formation of COPI-coated buds, but does not support scission. Chemical cross-linking of this Arf1 mutant restores vesicle release. These studies show that initial curvature of the bud is defined primarily by coatomer, whereas the membrane curvature modulating activity of dimeric Arf1 is required for membrane scission.

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Loss of the Cdc42-Interacting Protein, CIP4, Results In Murine Thrombocytopenia: insights into Wiskott-Aldrich Syndrome

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Background: Using the Src kinase Lyn as bait in yeast two-hybrid screen, we isolated Cdc42 interaction protein 4 (CIP4). CIP4 is a member of the F-BAR family of proteins, which generate membrane curvature and promote cortical actin polymerization. CIP4 possesses an SH3 domain that almost exclusively interacts with WASP and Dynamin, a GTPase involved in membrane scission. To better understand the physiologic role of CIP4, we made a CIP4 knockout mouse. We have reported that these mice display defects in clathrin-dependent and clathrin-

independent endocytosis (Feng et al **J Biol Chem** 285:4348, 2010). We now report that these mice have a thrombocytopenia comparable to that observed in WAS-/0 mice.
Hypothesis: Because of CIP4's interaction with WASP, we hypothesized that there diminished platelet production is due to WASP-dependent and Dynamin-dependent mechanisms.
Methods: We are using a CIP4 knockout mouse in the C57BL/6 background. We performed complete blood counts and bone marrow examination in the mice. We studied the CFU-MKs by using the collagen-based MegaCult system. We studied the megakaryocyte ploidy by flow cytometry. We assessed the in vitro ability of megakaryocytes to produce proplatelets. We are also performing biophysical studies of the plasma membrane in a human megakaryocytic cell line CHRF-288-11 with a lentiviral shRNA mediated knockdown of CIP4.
Results: Complete blood counts performed on C57BL/6 male mice between the ages of 3 and 6 months showed a decrease in platelets in CIP4-/- mice, comparable to WAS- mice. Interestingly, the CIP4-/-, WAS- mice displayed a more pronounced thrombocytopenia. No other defects were observed in hemoglobin and total leukocyte, absolute neutrophil, and absolute lymphocyte counts. Mean platelet volume was normal in CIP4-/- as in WAS-/0 mice. Bone marrow examination and scanning electron micrographs showed dysmorphology of the nuclei. There was no difference in numbers of CFU-MK, CFU-GEMM, or CFU-GM between CIP4-/- and wild-type mice. Propidium iodine staining of CD41+ bone marrow cells showed similar pattern of ploidy in wild-type and CIP4-/- mice. However, CIP4-/- megakaryocytes displayed fewer proplatelet protrusions. Proplatelet protrusions are due to membrane remodeling and elongation due to actin and microtubule polymerization. This effect is similar to our observation of fewer invadopodia in MDA-MB-231 breast cancer cells, which our lab recently reported (Pichot et al, **Cancer Research** 70:8347, 2010). In a megakaryoblast cell line which was infected with lentiviral shRNA to CIP4, decreased proplatelet protrusiuons were observed. Thus, there is a Lyn-Cdc42-CIP4-WASP pathway and a WASP-independent pathway involved in platelet biogenesis. Further biochemical characterization, including biophysical analysis will be presented.
Conclusions: These findings suggest that CIP4 may contribute to thrombocytopenia by affecting membrane remodeling and proplatelet protrusion stability.

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Manipulation of host cell membranes by *Salmonella* effector proteins

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Salmonella is a bacterial pathogen. Ingested bacteria cross the intestinal epithelial barrier and proliferate within epithelial cells and monocytes/macrophages. Intracellular replication takes place in a membrane-bound compartment called the *Salmonella*-containing vacuole (SCV). Infected cells are characterized by a profound reorganisation of late endocytic compartments. While lysosomal contents (i.e. lysosomal enzymes) tend to disappear from infected cells, lysosomal membrane glycoproteins (i.e. LAMP) accumulate on SCVs and on membrane tubular structures that extend from the SCV. These modifications involve the translocation into the host cell of bacterial effector proteins.
Among these effector proteins, SifA is required for the SCV stability and the formation of membrane tubular structures. Together with its eukaryotic target SKIP, SifA is also necessary for the removal of kinesin-1, which is recruited on the SCV membrane by the PipB2 effector.
We recently showed that the effector protein SopD2 is responsible for the SCV instability that triggers the cytoplasmic release of a *sifA*$^-$ bacterial mutant. Membrane tubular structures that extend from the SCV are the hallmark of *Salmonella*-infected cells, and until recently, these unique structures had not been observed in the absence of SifA. The deletion of *sopD2* in a *sifA*$^-$ mutant strain re-established membrane trafficking from the SCV and led to the formation of new membrane tubular structures, the formation of which is dependent on other *Salmonella* effector(s). Taken together, our data demonstrate that SopD2 inhibits the vesicular transport and the formation of tubules that extend outward from the SCV and thereby contributes to the phenotypes observed in absence of SifA.
The antagonistic roles played by SopD2 and SifA in the membrane dynamics of the vacuole, and the complex actions of SopD2, SifA, PipB2 and other unidentified effector(s) in the biogenesis and maintenance of the *Salmonella* replicative niche will be discussed.

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The non-receptor tyrosine kinase Fer is required for ErbB2-mediated tumorigenesis

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Fer is a ubiquitously expressed F-BAR containing cytoplasmic protein-tyrosine kinase which is activated upon engagement of numerous growth factor, cytokine, cell-cell and cell-matrix receptors and regulates membrane-cytoskeletal dynamics associated with cell polarity, cell migration and receptor signaling. In addition to C-terminal kinase and central SH2 domains, Fer has an N-terminal membrane-binding F-BAR domain implicated in vesicular trafficking. Here we provide *in vivo* evidence that Fer is pro-oncogenic. A kinase-inactivating knock-in mutation at the *fer* locus delayed tumor onset in a transgenic mouse model of ErbB2-mediated breast tumorigenesis. Tumor cells from Fer-deficient mice displayed accelerated EGF-induced endocytosis and enhanced MAPK signaling which correlated with elevated *in vivo* Erk activity. These observations suggest Fer promotes ErbB2-mediated tumorigenesis by regulating EGF-mediated endocytosis of EGFR/ErbB1 and ErbB2 receptors.

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Biomechanical remodeling of the microenvironment by stromal Caveolin-1 favors tumor invasion and metastasis

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Mechanotransduction, a key determinant of tissue homeostasis and tumor progression, is driven by intercellular adhesions, cell contractility and forces generated with the microenvironment, dependent on extracellular matrix composition, organization and compliance. Caveolin-1 (Cav1) favors cell elongation in 3D cultures and promotes Rho- and force-dependent contraction, matrix alignment and microenvironment stiffening through regulation of p190RhoGAP. In turn, microenvironment remodeling by Cav1-fibroblasts forces cell elongation. Cav1-deficient mice have disorganized stromal tissue architecture. Stroma associated with human carcinomas and melanoma metastases is enriched in Cav1-expressing carcinoma-associated fibroblasts (CAFs). Cav1 expression in breast CAFs correlates with low survival, and Cav1 depletion in CAFs decreases CAF contractility. Consistently, fibroblast expression of Cav1, through p190RhoGAP regulation, favors directional migration and invasiveness of carcinoma cells *in vitro*. *In vivo*, stromal Cav1 remodels peri- and intratumoral microenvironments to facilitate tumor invasion, correlating with increased metastatic potency. Thus, Cav1 modulates tissue responses through force-dependent architectural regulation of the microenvironment.

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Regulation of plasma membrane dynamics by I-BAR domain proteins

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Most BAR and F-BAR domain proteins sense/generate positive membrane curvature and are thus linked to the formation of plasma membrane invaginations during endocytic processes. In contrast, the I-BAR domain proteins IRSp53, IRTKS, MIM and ABBA generate/sense negative membrane curvature and are involved in the formation of plasma membrane protrusions in mammalian cells. Interestingly, the fifth mammalian I-BAR domain protein, which we named Pinkbar, does not tubulate phosphoinositide-rich membranes *in vitro*, but instead stabilizes planar membrane sheets through a mechanism that involves a relatively flat membrane-binding site of the domain and its oligomerization into a sheet-like structure *in vitro*. To elucidate how I-BAR, F-BAR and BAR domain proteins affect the distribution and dynamics of phosphoinositides, we have used a combination of fluorometric assays and live-imaging of giant unilamellar vesicles (GUVs). These experiments

revealed that all I-BAR/F-BAR/BAR domains tested induce the clustering of PIP_2. Importantly, at least the mammalian I-BAR domains and budding yeast BAR/F-BAR domains also significantly limit the diffusion of PIP_2 on GUVs. Thus, in addition to affecting the shape of membranes, I-BAR, F-BAR and BAR domains can regulate the distribution and dynamics of phosphoinositides at the plasma membrane.

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Inactivation of actin-binding protein affects elastic properties of eucaryotic cells

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The actin cytoskeleton is substantially modified in transformed cells, possibly accompanied with changes in the actin-binding proteins (Jordan and Wilson, 1998). As a result, cancer cells are more easily deformed than healthy cells. Recent studies have shown that benign cells are at least 70% stiffer than cancer cells (Cross et al, 2007). It has been suggested that the elastic properties of cancer cells play a major role in the metastatic process and that elastic properties such a deformability of the cells could be used as a marker for metastatic potential (Guck et al, 2005).
We studied the effect of inactivation of a major actin nucleator – formin on the elasticity of the cells. We compared Vero kidney epithelial cells extracted from an African green monkey, to the prostate cancer epithelial cell lines: DU-145 and PC3. We used AFM to measure the Young moduli as described in (Rizvi et al, 2009) with some modifications.
The Young moduli for normal cells were in the range of 2000 Pa, which was consistent with previous results for non-malignant cells (Faria et al, 2008). DU-145 and PC3 cancer cells exhibited much lesser Young moduli, comparing to the normal cells (40 to 42% of normal value). When we treated normal cells with 2 mkM solution of formin inhibitor SMIFH2 (Rizvi et al, 2009) for 14 hr, the apparent Young module decreased to the 30% of the value for normal cells. The successive investigation of cells in fluorescent confocal microscope, after labeling with rhodamine-stained phalloidin revealed that indeed the number of actin filaments was decreased in the treated cells, and they were mostly localized near the bottom of the cells. We observed also the increasing number of very small filopodia in the treated cells. Earlier we demonstrated that formins are upregulated in the tissues of prostate cancer patients (Sokolova et al, 2011). Taking into account the fact that decreasing of f-actin is often observed in cancer cells, we hypothesized that formins in prostate cancer tissue may be inactivated. The inactivation of actin nucleators leads to the changing the elastic properties of cancer cells.

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The F-BAR protein NOSTRIN is necessary for proper vascular development in zebrafish and mice

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To elucidate the function of the F-BAR protein NOSTRIN *in vivo* we carried out a morpholino-mediated knockdown (KD) of NOSTRIN in developing zebrafish embryos. The NOSTRIN KD resulted in vascular defects characterized e.g. by the irregular appearance of intersegmental vessles (ISVs) with wrong connections formed between neighbouring ISVs in both morphants, misshaped dorsal londitudinal anastomotic vessels (DLAV), hindbrain edema and cranial haemorraghing. The specificity of the vascular defects was demonstrated by a dose-dependent rescue of the phenotype by re-introduction of NOSTRIN by simultaneous injection of NOSTRIN mRNA together with the SH3 splice MO.
In vivo time-lapse microscopy of NOSTRIN morphants revealed delayed occurrence of ISV sprouts and their failure to grow dorsally in a directed fashion. This was characterized by leading cells, which remained at the level of the myoseptum, explored the surrounding area and established wrong connections with the neighbouring ISV. This was associated with a change of tip cell morphology from the characteristic elongated shape in control embryos to a stub like appearance in NOSTRIN morphants and a significant reduction of the length of tip cell filopodia. In addition we generated NOSTRIN knockout mice and studied the postnatal retinal angiogenesis. Confirming our findings in zebrafish, we found a significant reduction of the mean vascular radius in NOSTRIN KO mice at postnatal day 5 (P5) and P7. This was accompanied by a decrease of the number tip cells and filopodia.
Filopodia formation is critically controlled by the activity of small GTPases of the Rho family. Here we show for the first time that NOSTRIN interacts with Rac1 (via the NOSTRIN HR1 motif) and the Rac GEF SOS (via the

NOSTRIN SH3 domain and the proline-rich domain of SOS). NOSTRIN overexpression resulted in Rac1 activation, in contrast to NOSTRIN mutants unable to interact with either Rac1 or SOS. Finally, we could stimulate Rac activity in primary endothelial cells isolated from wt mice but not in cells from NOSTRIN knockout mice, confirming our previous findings that NOSTRIN is favourable for the activation of Rac. In this study we show for the first time that NOSTRIN is necessary for the co-ordinated vascular development in zebrafish and involved in postnatal retinal angiogenesis in mice. Taken together our results indicate a hitherto unknown important function of NOSTRIN for Rac1 activity during vascular development.

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The F-BAR protein, PSTPIP2, mediates membrane-cytoskeletal interactions in macrophage and osteoclast development and function

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The colony stimulating factor-1 (CSF-1) receptor tyrosine kinase (CSF-1R) controls macrophage and osteoclast production and function via the action of its ligands, CSF-1 and IL-34. Proline-, serine-, threonine-, phosphatase-interacting protein 2 (PSTPIP2), also known as macrophage F-actin-associated and tyrosine phosphorylated protein (MAYP), is an F-BAR domain-containing and actin-bundling protein, expressed predominantly in myeloid cells, that is rapidly tyrosine phosphorylated downstream of the CSF-1R. Several point mutations in the coding region of mouse *Pstpip2* lead to decreased PSTPIP2 expression and autoinflammatory disease with generalized osteopenia, reflecting its action as a suppressor of macrophage and osteoclast development and activation. In macrophages, PSTPIP2 controls CSF-1R-regulated morphological responses that include membrane ruffling, cell spreading, polarization, filopodia formation and chemotaxis. PSTPIP2 also regulates phagocytosis and the stimulated release of certain inflammatory mediators by macrophages. These results and the genetic and cell biological approaches being used to understand how PSTPIP2 regulates these diverse activities will be presented.

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Membrane fission can be driven by hydrophobic insertions and controlled by protein scaffolds: a model

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In the course of generating intracellular compartments membranes must undergo shaping consisting of two consecutive geometrical transformations: generation of curvature and remodeling of curvature by fission. We ask whether a single protein can drive the whole process of membrane shaping or whether the two transformations require different proteins. The two major mechanisms of membrane curvature generation by proteins are: membrane scaffolding by protein domains with an intrinsic curvature and insertion of hydrophobic protein regions into the membrane matrix. Here we use elastic models of lipid bilayers and protein scaffolds to demonstrate that the hydrophobic insertion mechanism can drive membrane fission in addition to membrane bending. We analyze quantitatively the conditions of membrane fission by this mechanism and demonstrate their

biological feasibility taking the epsin ENTH domain and the N-BAR domains of endophilin and amphiphysin as paradigm proteins. We predict that epsin will be more liable to promote membrane fission and we also predict a difference in the abilities of endophilin and amphiphysin to induce membrane fission. We propose that the suggested mechanism may underlie membrane remodeling by numerous proteins known to have a potential to bend membranes by the hydrophobic insertion mechanism.

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Molecular Mechanisms for Inducing and Sensing Membrane Curvature

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Recent work has illustrated the active role that proteins can play in shaping cellular membranes, but how proteins can induce a plethora of different membrane shapes is only poorly understood. Our work is aimed at understanding how proteins sense and induce different types of membrane curvatures on a molecular level. These mechanisms are studied in different membrane curvature-inducing systems, the BAR domain-containing proteins endophilin and amphiphysin, as well as the Parkinson's disease protein, α-synuclein. We find that each protein has the ability to induce different types of tubules that vary in shape and size and that can either be water filled bilayer tubes or cylindrical micelles. In addition, membrane curvature can also cause vesiculation as well as the formation of smaller, non-vesicular structures, which we readily observed in the case of α-synuclein. Using a combination of biophysical methods, including EPR spectroscopy and cryo electron microscopy, we find that the protein structure can vary significantly depending on which type of membrane curvature is induced. This is particularly the case for α-synuclein, where an extended helical structure mediates tubulation by wedging itself into the membrane, but where the smaller non-vesicular structures are stabilized by a bent, multi-helical structure. We also find that endophilin and amphiphysin have different modes of membrane binding depending on whether they cause tubulation or vesiculation. Our data suggest that different structural requirements need to be met for inducing different types of membrane curvature and that each of the proteins has sufficient conformational flexibility to accomplish this task. The precise protein structure and membrane curvature might, therefore, need to be controlled precisely and depend upon the cellular context. In the case of α-synuclein, we also find that membrane curvature induction can cause significant disruption of membrane integrity, a feature that might contribute to its toxicity *in vivo*.

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Membrane nanotubes: a biophysics tool for understanding the mechanical action of N-BAR proteins on membranes

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Membrane transport between intracellular compartments, entry or exit out of the cell, imply similar sequential events: membrane deformation and lipid/protein sorting during the formation of the transport intermediate (vesicle or tube), fission from the donor compartment, transport and eventually fusion with the acceptor membrane. The mechanisms behind these biological processes of membrane transformation are actively studied both in the cell biology and the biophysics contexts. Model membranes are convenient systems to investigate mechanism involved in cell trafficking. Indeed, they are composed of a very limited number of components compared to cell membranes, their mechanics can easily be interpreted and comparison with physics models is thus facilitated (1,2). Among them, membrane nanotubes with a controlled diameter (15-500 nm) pulled out of Giant vesicles (GUV) are very convenient tools to address the role of curvature in trafficking events (3) and to measure mechanical effects due to protein binding using optical tweezers (4) .
As an example of this type of approach combining in vitro experiments and theoretical modeling, I will present our results on amphiphysin 1, which is implicated in clathrin-mediated endocytosis. It contains a N-BAR membrane-binding domain that senses and generates membrane curvature. We can summarize its behaviors

according two regimes depending on the protein density on the nearly-flat GUV. At low densities, the distribution of proteins and the mechanical effects induced are described by a model based on *spontaneous curvature* induction. The tube radius and the force needed to hold the tube are modified by protein binding but still depend on membrane tension. In the dilute limit, when practically no proteins were present on the vesicle, no mechanical effects were detected, but a strong protein enrichment proportional to curvature was seen on the tube. At high densities, the radius and force are independent of tension and vesicle protein density, resulting from the *formation of a scaffold* around the tube. For the entire density range, protein was found to be more enriched on the tube than on vesicle, showing a concomitant *curvature-sensing* ability. Our approach shows that the strength of curvature sensing and mechanical effects on the tube depends on the protein density (5).

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Essential elastic and shape parameters govern the dynamics and energetics of dynamin-mediated membrane fission

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Dynamin is a GTPase that polymerizes into helical collars at the neck of endocytic buds, and constricts upon GTP hydrolysis in order to release free vesicles. Several hypotheses are currently being debated to understand how this polymerization and conformational change are promoting membrane fission in a controlled manner.

I'll show recent results where we have investigated the lipid membrane parameters influencing the fate of dynamin reaction. By using templates consisting of thin lipid tubules extracted by optical tweezers from Giant Liposomes maintained in micropipettes, we studied the mechanical parameters controlling membrane fission. We showed that if the curvature of the tube triggers polymerization of dynamin (spontaneous nucleation is observed at radii below 20nm), the saddle-like shape of endocytic buds strongly favors the dynamin reaction. As well, high membrane tension intensively reduces both the stochasticity and the average time it takes for fission. Our findings support the idea that the high curvature of the neck and its saddle-like shape are respectively triggering the nucleation of the polymer and the membrane break. Also, as expected from our previous findings, membrane tension is a primordial parameter of fission efficiency.

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ESCRT-III regulation, assembly and membrane remodeling

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Endosomal sorting complexes required for transport (ESCRTs) catalyze multivesicular body biogenesis, distinct steps in cell division and budding of some enveloped viruses such as HIV-1. Out of the five complexes constituting the ESCRT machinery only members of ESCRT-III and the VPS4 complex are essential for all ESCRT-catalyzed cellular processes because they catalyze membrane scission. Mammalian cells express 12 ESCRT-III members, which are cytosolic and transiently recruited to cellular membranes upon activation. Membrane interaction then induces polymerization required for membrane remodeling steps. We present structural and functional data on the regulation of ESCRT-III CHMP3 by the ubiquitin hydrolase AMSH which

can interact with ESCRT-III polymers. In contrast a new regulator of ESCRT-III CHMP4, CC2D1A targets CHMP4 monomers and prevents CHMP4B polymerization. Their effect on HIV-1 budding will be discussed. We will further present structural models of ESCRT-III polymers and show that tubular structures of CHMP2A-CHMP3 are composed of linear filaments made up by monomers. We present an interaction map of ESCRT-III members based on SPR and discuss their potential for complex formation. Finally we show that CHMP2B expression in HEK cells leads to plasma membrane tube formation controlled by CHMP4 and VPS4. Structural analysis of these membrane tubes demonstrates that CHMP2B forms helical filaments associated with membrane, which follow the same structural principles as the CHMP2A-CHMP3 tubular structures assembled *in vitro* (without membrane). Furthermore CHMP2B membrane tubes reveal sites of constriction that indirectly support the dome-like model for membrane scission. An integrated model of ESCRT-III assembly will be discussed.

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The organization and remodeling of biological membrane

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As eukaryotic cytoplasm evolved, the localization of enzymatic processes to membranes provided many organizational advantages, including higher local concentrations within organelles, reduction of dimensionality for diffusion in the plasma membrane, and segregation of specialized components. On the plasma membrane, asymmetric lipid provided for both signaling through lipid redistribution and differential energy barriers for cell and organelle fusion. Our recent work in membrane remodeling and plasma membrane organization will be discussed from the perspective of both physiological and pathological phenomena. Systems will include the development and release of the newly replicated malaria parasite Plasmodium falciparum from human red blood cells, assembly of the influenza virus on the plasma membrane, synaptic vesicles of the Woods Hole squid, membrane damage and repair in muscle and brain, and the acrosomal exocytosis of the sea urchin sperm and the sperm-egg fusion of fertilization.

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EHD2 remodels caveolae membranes via specific ATP-driven targeting and oligomerisation

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EHD2 belongs to the Eps15 homology (EH) domain protein family of dynamin-related ATPases involved in membrane remodelling in the endosomal system. EHD2 dimers oligomerise into rings on highly curved membranes resulting in stimulation of the intrinsic ATPase activity. Here, we report that EHD2 is specifically and stably associated with caveolae at the plasma membrane and not involved in clathrin mediated endocytosis or endosomal recycling as previously suggested. EHD2 interacts with cavin1 and the membrane association of EHD2 is dependent on cavin1 and intact caveolae integrity. While the EH domain of EHD2 is dispensable for targeting, we identified a loop in the GTPase domain required for caveolae localisation. EHD2 overexpression promotes caveolae turnover and ATPase deficient mutants of EHD2 cause tubulation and distortion of caveolin positive membranes. Thus, following the identification of caveolin and cavins, EHD2 constitutes a third structural component of caveolae involved in the shaping of this organelle.

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Structure, oligomerization and mechanism of dynamin superfamily proteins

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GTPases of the dynamin superfamily remodel cellular membranes in response to nucleotide binding and hydrolysis. The molecular details of membrane interaction and the role of nucleotide-dependent changes for the function of these proteins are just emerging. Here, I present structural data on the dynamin-related proteins EHD2 [1] and MxA [2] which shed light on the mechanism of oligomerisation and the mechano-chemical function of these proteins. EHD2 oligomerizes via two distinct interfaces in the GTPase domain resulting in ring-like oligomers. Using electron paramagnetic resonance studies, we show that not only the tips of the helical domains but also the amino-terminus contribute to lipid binding. Furthermore, the Eps15 homology domains of EHD2 might switch from the top of the GTPase domain, as found in the crystal structure, to the side of the GTPase domain and also participate in membrane binding. Another mode of oligomerisation is found in the antiviral MxA GTPases which oligomerises via the helical stalk region to form ring-like structures. Furthermore, the GTPase domains of MxA might contribute to oligomerisation by connecting neighbouring rings. This assembly mode suggests a mechanism for the mechano-chemical function which is consistent with previous models for dynamin function. Finally, I will show how structural information obtained for MxA can be employed to obtain insights into structure and function of the dynamin GTPase.

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Three distinct pathways by which curvature modulates the structure and function of membranes

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To date we have established exhaustive correlations between the lipid composition of membranes and its impact on membrane properties and protein function. In addition to composition the shape of cellular membranes appears to be a well-conserved phenotype in evolution. Nevertheless we largely ignore what are the consequences of membrane shape/curvature to biological functions that make it so critical for sustaining life. The lack of information on the significance of membrane shape has predominantly been due to the absence of reliable assays that allow us to perform systematic experiments as a function of membrane shape/curvature. We have recently demonstrated the possibility to construct a high throughput array of unique nanoscale membrane curvatures. The assay is based on unilamellar liposomes of different diameters (30 nm to 700 nm), and therefore curvature, that are immobilized on a surface at dilute densities allowing for imaging of single liposomes with fluorescence microscopy.
Here I will discuss published and unpublished data on several important classes of biomolecules whose localization, structure or oligomeric state exhibited dramatic curvature dependence including i) GPCRs and ii) membrane anchored proteins.

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3 **EMBO Journal,** 2009, 28 (21), p. 3303 *Amphipathic motifs in BAR domains are essential for membrane curvature sensing*. V. K. Bhatia, K. L. Madsen, P. Y. Bolinger, P. Hedegård, U. Gether, D. Stamou.
4 **Proceedings of the National Academy of Sciences. 2009**. 106 (30): p. 12341 *Quantification of nano-scale intermembrane contact areas using fluorescence resonance energy transfer*. P. M. Bendix, M. S. Pedersen and D. Stamou.

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Rab GTPases and actin-based motors

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Rab GTPases are key regulators of vesicular transport and membrane trafficking in eukaryotic cells. To fulfill their tasks, Rab GTPases interact with a wide variety of effectors, including actin- and microtubule-based molecular motors. Here, we illustrate the functional interactions between several Rab GTPases and two classes

of myosins, myosin II and myosin V. The Golgi-associated Rab6 GTPase can recruit myosin II on Golgi/TGN membranes to control the fission of Rab6 positive transport carriers. Rab8/Rab11 can recruit myosin V on recycling endosomes and likely participate in their tethering to actin filaments. In addition, myosin V is able to interact though non-overlapping domains with at least eight Rab GTPases, suggesting the existence of a complex network of interactions required to sustain intracellular transport and membrane dynamics at the Golgi/TGN/recycling endosomes interface.

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Coupled dynamics of actin filaments and membranes in motile processes: a reconstitution approach

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Cell movements are organized by the concerted turnover of actin filaments in well defined self-organized modules that develop protrusive, adhesive and contractile forces.
Force is produced by site-directed polarized assembly of actin filaments against the membrane. Macroscopic scale deformation of membranes result from molecular chemical reactions in which the initiation and growth of actin filaments is coupled to membrane dynamics by signal-responsive protein machineries. Two main machineries are known. The WASP family proteins use Arp2/3 complex machinery to branch filaments at the membrane and generate either protrusive forces in lamellipodium extension, or membrane scission and vesicle formation in traficking and endocytic processes. The formin family, on the other hand, initiate processive assembly of actin filaments from profilin-actin complex in filopodia, bundles and various more complex processes.
These elementary modular actin arrays often combine with other proteins like tropomyosin and myosin, thus establishing higher spatial order in cells like lamellar arrays enriched in tropomyosin, contractile arcs. Similar combination of concerted actin arrays are responsible for the translocation of the spindle in asymmetric cell division or the formation of the immunological synapse or the neural growth cone.
We propose a reconstitution approach using functionalized beads and giant liposomes propelling by actin assembly in chemically controlled solutions to address the following issues : 1) How is the dynamics of actin assembly by WASP/Arp2/3 and formins coupled to membrane dynamics ? 2) How is spatial order and segregated distribution of various proteins established in actin arrays ?
We show that the catalytic mechanism of filament branching controls the distribution and lateral mobility of WASP at the membrane, and that the kinetics of filament debranching controls the enriched distribution of tropomyosin on filaments at the rear of the lamellipodium. Thus simple physical-chemical mechanisms inherent to regulated actin assembly define spatial order. Further projects address the mechanism of establishment of higher spatial order in reconstituted self-organized systems.

References

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Cortical forces in cell shape changes and tissue morphogenesis

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Cortical forces drive a variety of cell shape changes and cell movements during tissue morphogenesis [RAU 2008, RAU 2011]. While the molecular components underlying these forces have been largely identified, how they assemble and spatially and temporally organize at cell surfaces to promote cell shape changes in developing tissues are open questions. I will present how cortical forces emerge from the dynamics of actomyosin networks

in interaction with adhesion complexes during early embryogenesis. I will focus on the elongation of *Drosophila* melanogaster embryos, which results from polarized cell neighbour exchanges. I will emphasize the pulsatile nature of force generation, the role of actomyosin flows [RAU 2010] and that of E-cadherin distribution in the remodelling of cell-cell contacts.

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14-3-3γ regulates CtBP1-S/BARS-mediated fission of post-Golgi carriers

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Membrane fission is required during the formation of intracellular transport carriers, a central process in membrane trafficking. Some fissioning mechanisms involve dynamin, and we have shown a fundamental role for CtBP1-S/BARS (BARS) at the Golgi complex and in fluid-phase endocytosis, thus defining a novel dynamin-independent fissioning machinery. To identify BARS-interacting proteins and to define their roles in BARS-induced fission, we used affinity chromatography, mass spectrometry and Western blotting. One such protein is the γ isoform of the 14-3-3 proteins, which interact with phosphatidylinositol 4-kinase (PI4K) IIIβ in both mammals and yeast. PI4KIIIβ and phosphatidylinositol 4-phosphate are fundamental to the organisation of the Golgi complex and to post-Golgi transport in mammalian cells. In pull-down assays, 14-3-3γ binds both BARS and PI4KIIIβ, although BARS does not bind PI4KIIIβ directly. In COS7 cells expressing a GFP-tagged temperature-sensitive mutant of the G protein of vesicular stomatitis virus (VSVG) as a cargo reporter, RNA interference against 14-3-3γ inhibited *trans*-Golgi network (TGN)-to-plasma membrane transport of VSVG (by 60%). Here, a decrease in post-Golgi transport carriers was accompanied by formation of long VSVG-containing tubules (5-30 μm) from the TGN. These tubules elongated (and retracted) at 0.5 μm/s, consistent with microtubule-based motility. Thus, 14-3-3γ is involved in BARS mediation of TGN-to-plasma membrane transport through control of the formation/ fissioning of constitutive post-Golgi carriers. Förster resonance energy transfer analysis shows that at the Golgi complex, 14-3-3γ dimers bridge the fission-inducing protein BARS with PI4KIIIβ, and that this complex is stabilised by phosphorylation of Ser294 in PI4KIIIβ and Ser147 in BARS by the kinases PKD and PAK, respectively. Disruption of this complex inhibits fissioning of elongating post-Golgi carrier precursors, thus providing a functional link from PI4KIIIβ-dependent tubulation/ post-Golgi carrier formation to BARS-induced fissioning. Regulated assembly of this complex provides new insights into the molecular organisation of post-Golgi carriers.

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Visualizing vesicle and virus budding by electron microscopy

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The budding of coated vesicles and membrane viruses can be studied using new electron microscopy methods. I will illustrate how cryo-electron tomography and sub-tomogram averaging can describe the structure and arrangement of protein coats in situ on vesicles and viruses, and how correlative light and electron microscopy can identify intermediates in budding processes. I will present our results from applications of these methods to COPI, clathrin and HIV Gag mediated budding.

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Molecular Determinants of Membrane Fusion and Fission

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

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POSTER PRESENTATIONS

COMMUNICATIONS AFFICHEES

Sunday, September 25

Dimanche 25 septembre

20:30 - 21:30

Poster session I

Session I de communications affichées

Poster board number		*Abstract*
1	**ÅHLUND Monika**	p. 83
5	**AHMED Sohail**	p.
7	**AVULA Tejakalyan**	p.
10	**BARELLI Hélène**	p.
13	**BERGAN Jonas**	p.
17	**BIGAY Joelle**	p.
19	**BISI Sara**	p.
22	**BRODIN Lennart**	p.
25	**CHASSEROT-GOLAZ Sylvette**	p.
27	**CHITU Violeta**	p.
32	**CHWASTEK Grzesiek**	p.
36	**COUDRIER Evelyne**	p.

21:30 - 22:30

Poster session II

Session II de communications affichées

Poster board number		*Abstract*
3	**COWLING Belinda**	p.
8	**DISANZA Andrea**	p.
12	**DUMENIL Guillaume**	p.
15	**ECHARRI Asier**	p.
20	**EL FAR Oussama**	p.
24	**GAUTREAU Alexis**	p.
29	**GINIATULLINA Asiya**	p.
31	**GKOURTSA Areti**	p.
34	**GOLINELLI Marie-Pierre**	p.
38	**HATZAKIS Nikos**	p.
40	**JEMAIEL Aymen**	p.
41	**JULLIÉ Damien**	p.

Monday, September 26

Mardi 26 septembre

17:30 - 18:15

Poster session III

Session III de communications affichées

Poster board number		*Abstract*
2	**KOUTSOPOULOS Olga**	p.
9	**KOVAČEVIĆ Igor**	p.
16	**LENNON-DUMENIL Ana-Maria**	p.
23	**MAZZOLINI Julie**	p.
26	**MOREN Björn**	p.
28	**PAGLIUSO Alessandro**	p.
33	**PERINI Enrico**	p.
35	**PYKALAINEN Anette**	p.
39	**RODGERS William**	p.

18:15 - 19:00

Poster session IV

Session IV de communications affichées

Poster board number		*Abstract*
4	**ROMPF Maria**	p.
6	**ROSENDALE Morgane**	p.
11	**SANCHEZ-BARRENA Maria Jose**	p.
14	**SOYER Magali**	p.
18	**STANISHNEVA-KONOVALOVA Tatiana**	p.
21	**TAKEDA Tetsuya**	p.
30	**YAMADA Ayako**	p.
37	**ZHAO Hongzia**	p.
42	**ZHANG Sicai**	p.

A role for GRAF1-mediated endocytosis at cell-matrix adhesion sites

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The rho GTPase activating protein GRAF1 mediates membrane remodelling during formation of the prevalent, tubulovesicular, clathrin-independent carriers (CLICs) responsible for lipid-anchored receptor endocytosis. We show that active cdc42 induces GRAF1-dependent CLICs. Our results also reveal that GRAF1 interacts with a network of endocytic and adhesion proteins. Furthermore, at a cellular level, the protein is found at smaller vinculin-positive structures reminiscent of what have previously been described as podosome-like adhesions. Promoting the formation of such podosome-like adhesions via manipulation of cdc42-activity or drug-induction of adhesion site turnover, results in clustering of endocytic cargo in these GRAF1- and vinculin-positive structures. Our data also show that GRAF1 activity is up-regulated in spreading cells and that uptake via CLICs is concentrated at the leading edge of migrating cells. Interestingly, depleting cells of GRAF1, and thereby inhibiting CLIC generation, causes profound defects in cell spreading and migration. We therefore propose a role for GRAF1 in remodelling of membrane microdomains at adhesion sites to facilitate membrane turnover during cell morphology changes.

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The I-BAR domain protein IRSp53 interacts with and utilizes mDia1 for filopodia and neurite formation in N1E115 cells.

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IRSp53 plays a major role in inducing morphological change downstream of its GTP binding protein effector Cdc42. In N1E115 cells the Cdc42-IRSp53 complex has been shown to drive filopodia and neurite formation. IRSp53 possesses an I-BAR domain, a Cdc42 binding site and a SH3 domain. A number of proteins have been shown to bind the IRSp53 SH3 domain and this includes; Mena, N-WASP, Eps8, WAVE1, WAVE2, and mDia1. The molecular mechanism of filopodia formation through IRSp53 is to couple I-BAR domain mediated membrane protrusion with SH3 domain mediated actin dynamics.
In this presentation we show that IRSp53 synergises with mDia1, but not mDia2, in both filopodia formation and neurite outgrowth. IRSp53 and mDia1 colocalise with each other in filopodia while IRSp53 and mDia2 colocalise in endsome-like structures. FRET experiments show that IRSp53 can bind both mDia1 and mDia2 in vivo, but only the former protein binds IRSp53 in filopodia. Knockdown of mDia1 but not mDia2 inhibited filopodia formation. Taken together, these data suggest that mDia1 plays a role in filopodia and neurite formation downstream of the Cdc42-IRSp53 complex.

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BAR domain proteins in fission yeast

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The fission yeast *Schizosaccharomyces pombe* is phylogenetically divergent from *Saccharomyces cerevisiae*, *Candida albicans*, and *Ashbya gossypii,* and is characterized by a cylindrical rod-like shape and a medial septum dividing the cell. These features make it an ideal organism to study cell polarity and cell morphogenesis
The *S. pombe* genome encodes three proteins with a Src Homology 3 (SH3) domain that lack a homolog in the three other yeast species mentioned: NonHom1, NonHom2, and NonHom3. Interestingly, these three SH3 proteins also appear to possess a Bin-Amphyphysin-Rvs (BAR) domain. BAR domains form homodimers or heterodimers that bind preferentially to membranes of high curvature. In yeasts, two different types of BAR domains are known to occur, F-BAR and N-BAR domains, and NonHom1 appears to contain an F-BAR domain whereas NonHom2 and NonHom3 appear to contain an N-BAR domain.
F-BAR proteins usually form homodimers, and we have confirmed in yeast two-hybrid analysis that the F-BAR domain of NonHom1 interacts with itself, similar to the homodimerization shown for the known F-BAR domain of Imp2. By contrast, N-BAR domains often form heterodimers, and we confirmed in yeast two-hybrid analysis that the N-BAR domains of NonHom2 or NonHom3 fail to interact with themselves. Furthermore, these N-BAR

domains failed to interact with each other, suggesting that they both interact with other, yet unidentified, N-BAR proteins. We are setting up a yeast two-hydbrid screen to identify their binding partners. As a positive control, we showed that the N-BAR domains of *S. pombe* Rvs161 and *S. pombe* Rvs167 interact in yeast two-hybrid analysis. Interestingly, we observed a yeast two-hybrid interaction between the N-BAR domains of *S. pombe* Rvs161 and *S. cerevisiae* Rvs167, indicating that the dimerization interface between these Rvs proteins is conserved in evolution.
To study the NonHom proteins in greater detail, we expressed their BAR domains as fusion protein in *E. coli* and successfully obtained soluble protein for each. In *in vitro* binding studies, we wish to confirm homodimization of the F-BAR domain of NonHom1. Furthermore, we will use the soluble BAR domains to study their interaction with curved membranes in liposome co-sedimentation assays.

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Exploring the surface features of subcellular membranes by fluorescent probes harboring specific amphipathic helices

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Cellular organelles differ in lipid composition. Thus, some rare phosphoinositides act as specific markers of compartments. For example, PI(4)P is mostly found at the trans Golgi region, whereas PI(4,5)P2 is more specific of the plasma membrane. But in addition, differences in the bulk lipid composition probably change the physical and chemical properties of the surface of membrane- bound organelles. To probe this, we have designed constructs containing a fluorescent moiety (e.g. GFP), a central coiled-coil region and two N-terminal amphipathic helices acting as sensor of the surface of cellular organelles. Because amphipathic helices are sandwiched between the polar head group and the acyl chains of lipids and because they interact simultaneously with many lipids, they are ideally positioned to integrate the complex chemistry (lipid packing, charge density) of the interfacial region of cellular membranes. A few examples based on three amphipathic helical motifs (ALPS, a-synuclein, Sop20) will be presented.

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The role of globotriaosylceramide in trafficking of Shiga toxin

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Shiga toxin is a bacterial toxin which is endocytosed into target cells upon binding to the glycosphingolipid globotriaosylceramide (Gb3). Gb3 consists of three sugar units (Gal-Gal-Glc) attached to an invariable sphingosine backbone and an N-amidated fatty acyl chain, which varies in both length and saturation, thereby giving rise to different species of Gb3. After internalization the toxin is sorted from endosomes to the *trans*-Golgi network and further to the ER, where the toxic subunit is translocated to the cytosol. We have previously found inhibitors of glycosphingolipid synthesis to mediate resistance to Shiga toxin, even when there is still a considerable amount of Gb3 available for binding and endocytosis (1). This indicates that Gb3 species may differ in their ability to bind and mediate retrograde transport of Shiga toxin. To further investigate the fate and role of these different species we have investigated Shiga toxin transport in cells where the activity of the Gb3-producing enzyme Gb3 synthase has been inhibited. When Gb3 synthase is knocked down by siRNA for as short as 24 hours, there is a strong protection against the Shiga toxin cytotoxicity, accompanied by only a moderate reduction in binding. This suggests that there are additional effects on the intracellular trafficking of the toxin, and supports the notion that removal of a minor fraction of Gb3 can confer a strong protection against Shiga toxin. Interestingly, MS analysis of whole cell lipids revealed that the siRNA treatment reduced all Gb3 species to a similar level, and this was accompanied by a corresponding increase in LacCer species and an unchanged level of GlcCer.

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Study of the mammalian sterol transporters OSBP1 and ORP9L

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We found that Kes1p/Osh4p a yeast protein of the Osh/Orp family was able to recognize membrane curvature via an ALPS motif (1) and more recently we demonstrated its ability to exchange sterol for PI(4)P between membrane (2). Based on these results, we suggest that Osh4p likely transport sterol from the ER to the trans-Golgi and transport PI(4)P in the opposite way.
In mammals, at least 12 different proteins contain a sterol-binding domain akin to Osh4p as well as additional domains, but none of them have an ALPS motif. For example, OSBP1 and ORP9L, known to be important for the regulation of cellular cholesterol homeostasis in the cell, have a long N-terminal sequence containing a PH domain, a dimerization domain and a FFAT motif. The PH domain is specific for both PI(4)P and Arf1-GTP, and allows interaction with the Golgi membrane (TGN), while the FFAT motif specifically recognizes the VAP-A protein of the ER. The mode of activation/regulation of these two proteins is largely unknown. Our project is to identify for each protein its membrane binding capacity and its ability to sense or transport sterol. We aim for example to reconstitute in vitro the sterol transport activity of these proteins between donor and acceptor membranes of defined composition (liposomes). One of our objectives is to see how the activity of OSBP and ORP9 is dependent on PI(4)P and /or Arf1(GTP). Preliminary results will be shown.

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Regulation of Synaptic Vesicle Budding and Dynamin Function by an EHD ATPase

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Eps15 homology domain-containing proteins (EHDs) are conserved ATPases implicated in membrane remodelling. Recently, EHD1 was found to be enriched at synaptic release sites in rat brain (Wei et al 2010), suggesting an involvement in the trafficking of synaptic vesicles. We have now investigated the role of an EHD1/3 ancestral ortholog in the lamprey giant reticulospinal synapse. EHD1/3 was detected by immunogold at endocytic structures adjacent to release sites. In antibody microinjection experiments, perturbation of EHD1/3 inhibited synaptic vesicle endocytosis and caused accumulation of clathrin-coated pits with atypical, elongated necks that were covered with helix-like material containing dynamin. To test whether EHD may directly interfer with dynamin function we used fluid supported bilayers (SUPER templates). We found that EHD1/3 strongly inhibited vesicle budding induced by dynamin in the constant presence of GTP (and ATP). It also inhibited dynamin-induced membrane tubulation in the constant presence of GTPγS, a phenomenon linked with dynamin helix assembly. Both inhibitory effects were relieved when ATP was replaced with ATPγS. EHD1/3 and dynamin did not appear to interact in solution, but using FRET we observed an interaction on the SUPER template membrane. Our *in vivo* results demonstrate an involvement of EHD1/3 in clathrin/dynamin-dependent synaptic vesicle budding. Based on our *in vitro* observations we speculate that EHD may act to limit the formation of long, improductive dynamin helices, thereby promoting vesicle budding.

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Annexin A2 mediates actin filament aggregation to form lipid microdomains at exocytotic sites in neuroendocrine cells

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Annexin A2 is a calcium and lipid binding protein involved in exocytosis in neuroendocrine cells. In chromaffin cells, this protein is involved in the formation and/or stabilisation of the lipid microdomains required for structural and spatial organization of the exocytotic machinery [1]. To understand how annexin A2 promotes

aggregation of lipids in the plasma membrane, we focussed on the role of actin in this process. Indeed annexin A2 is able to interact with actin to form a membrane-cytosqueleton complex and modulate membrane organisation [2, 3]. Immunolabelling analysed by electron microscopy showed that actin was present at the level of lipid microdomains formed at exocytotic sites and that latrunculin, a drug which depolymerises actin, inhibited the coalescence of lipid domains. To directly link annexin A2 with this observation, annexin A2 mutants which do not bind and aggregate actin were generated. The expression of these mutated proteins in chromaffin cells inhibited the formation of exocytotic sites, suggesting that the aggregation of actin microfilament by annexin A2 is required for secretion. Finally Withaferin A, which specifically activates the cross-linking of F-actin by annexin A2 [4] was tested. In chromaffin cells treated with Withaferin A, the secretion of catecholamines and the formation of lipid domains were increased. Moreover, at the ultrastructural level, Withaferin A affected the structure of actin filaments present in the vicinity of docked granules. Thus, our results suggest that annexin A2 may aggregate actin microfilaments associated with chromaffin granules to induce the coalescence of lipid microdomains required for exocytosis.

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Structural requirements for the negative regulation of osteoclast differentiation by PSTPIP2

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The concerted action of colony stimulating factor-1 (CSF-1) and RANKL drive the expression of osteoclast-specific genes and the fusion of mononuclear precursor cells in the generation of multinucleated bone-resorbing osteoclasts. Macrophage F-actin-associated and tyrosine phosphorylated protein (MAYP), also known as proline- serine- threonine- phosphatase-interacting protein 2 (PSTPIP2), is selectively expressed in the myeloid lineage and becomes rapidly tyrosine phosphorylated following activation of the CSF-1 receptor (CSF-1R) in myeloid cells. Mutations in *Pstpip2* lead to autoinflammatory disease and osteoporosis significantly contributed to by the expansion of myeloid precursors. However, osteoclastogenesis is also accelerated and increased in cultures of purified osteoclast precursors isolated from PSTPIP2-deficient mice. To understand how PSTPIP2 modulates osteoclast differentiation, we developed immortalized, PSTPIP-2-deficient, osteoclast precursor cell lines in which to study PSTPIP2 structure/function by retroviral reconstitution. PSTPIP2 expression was reconstituted in these cells using retroviruses encoding either wild type PSTPIPIP2, a mutant unable to interact with PEST-type phosphatases, an F-BAR domain mutant unable to interact with membrane phospholipids, or a mutant that is not tyrosine phosphorylated following the activation of Src family kinases by CSF-1R. Both an intact F-BAR domain and regulation of PSTPIP2 by tyrosine phosphorylation are essential for its ability to suppress tartrate-resistant acid phosphatase (TRAP) expression and osteoclast precursor fusion. In contrast, interaction with PEST-type phosphatases is important only for suppression of TRAP expression. Our results identify PSTPIP2 as an essential limiting factor of osteoclast differentiation acting downstream of the CSF-1R.

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Interactions of BAR proteins with lipid membranes

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BAR proteins constitute a big group of membrane binding proteins which do not only show high affinity to membranes, but also enhance bilayer deformation and tubulation. It was shown recently, that BAR proteins are important players in endocytosis, filopodia formation and rearrangement of the actin cytoskeleton [1,2,3]. Though the crystal structure of several BAR proteins has been resolved, many aspects of action of these proteins are unknown [4]. The aim of our work is to gain knowledge about the role of the lipid bilayer in BAR-induced membrane transformation. Instead of so far used methods like electron microscopy and biochemical assays, we apply different artificial membrane systems which can be comfortably studied by high-resolution fluorescence

microscopy. Such experimental approaches allow real-time monitoring of changes in the system and help us to better understand the whole process of BAR protein binding and consecutive membrane deformation. Our main focus is to establish an assay in which the membrane deformation can be quantitatively characterized. Moreover, the usage of an artificial membrane system allows us to easily control and change experimental conditions like pH, ionic strength of the buffer, and the lipid composition of the membrane. We strongly believe that such a system will become an excellent tool in the study of BAR and other membrane sculpting proteins.

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Myosin 1b promotes the formation of post-Golgi carriers by regulating actin assembly and membrane remodelling at the trans-Golgi Network

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The function of organelles is intimately associated with rapid changes in membrane shape. By exerting force on membranes, the cytoskeleton and its associated motors are major players in membrane remodelling. Actin and myosin 1 have been implicated in the invagination of the plasma membrane during endocytosis. However whether myosin 1 and actin contribute to the membrane deformation that gives rise to the formation of post-Golgi carriers is unknown.
Our results demonstrate that a pool of Myo1b located at the Trans Golgi Network (TGN) controls the TGN exit of mannose 6 phosphate receptor (MPR) and neurotrophin receptor p75 (p75) but not of a GPI-anchored protein. Morphogenesis of TGN transport carriers comprises the formation of tubular-carrier precursors, their extension and fission. By using live cell imaging and siRNA we showed that depletion of the Arp2/3 complex and depletion of Myo1b both, inhibit the formation of tubular-carriers precursors and post-Golgi carriers. Furthermore we observed that detection of the actin foci in the perinuclear region depends on Myo1b expression. Thus Myo1b and F-actin foci promote the formation of tubular-carrier precursors involved in MPR and P75 trafficking. We observed that Myo1b motor activity was required for the formation of F-actin foci and for the steady state MPR distribution. Altogether, our work reveals a new function for myosin 1b that is to couple actin assembly to organelles and control membrane deformation leading to the formation of transport carriers. These data suggest that Myo1b actively tethers and orients polymerising F-actin to the TGN membrane and generates a force leading to TGN membrane deformations (Almeida *et al.* Nat. Cell Biol. in press). We presently study the role of the other myosins (myosin II, myosin VI, Myosin 18A and myosin V) that contribute to different step of the membrane traffic at the TGN on the distribution of the actin foci.

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Altered Dynamin 2 expression *in vivo* provides new insights into the role of Dynamin 2 in skeletal muscle development, maintenance and disease

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Dynamin 2 (DNM2) is a large GTPase implicated in many cellular functions including cytoskeleton regulation and endocytosis. Whilst ubiquitously expressed, DNM2 was found mutated in two genetic disorders affecting different tissues: Autosomal Dominant Centronuclear Myopathy (ADCNM; skeletal muscle) and peripheral Charcot-Marie-Tooth neuropathy (CMTDIB; peripheral nerve).

To gain insight into the function of DNM2 in skeletal muscle and the pathological mechanisms leading to ADCNM, we introduced DNM2 wild-type or R465W (RW-DNM2), the most common ADCNM mutation, into adult wild-type mouse skeletal muscle by intramuscular Adeno-Associated Virus (AAV) injections. We detected altered localization of RW-DNM2 in mouse muscle. Several ADCNM features were present in RW-DNM2 mice; fibre atrophy, nuclear mislocalization, and altered mitochondrial staining, with a corresponding reduction in specific maximal muscle force. The sarcomere and triad structures were also altered. We report similar findings in muscle biopsies from an ADCNM patient with the R465W mutation. In addition, expression of wild-type DNM2 induced some muscle defects, albeit to a lesser extent than RW-DNM2, suggesting that the R465W mutation has enhanced activity *in vivo*. Therefore we showed the R465W DNM2 mutation acts in a dominant manner to cause ADCNM in adult muscle, and the disease arises from a primary defect in skeletal muscle rather than secondary to peripheral nerve involvement. It also suggests that DNM2 has an important role in the maintenance of muscle structure as adult muscles were injected.
To further decipher the function of DNM2 in skeletal muscle, we have created DNM2 knockout mice. Complete knockout of DNM2 in all tissues was lethal before embryonic day 12. Therefore we have developed a muscle-specific knockout mouse for DNM2 and have characterized muscle-specific isoforms of DNM2 in this tissue. Preliminary results will be presented. We conclude DNM2 plays important roles in both development of mouse skeletal muscle, and maintenance of adult muscle fibres.

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The I-BAR containing protein IRSP53 regulates filopodia formation by driving VASP clustering at the plasma membrane

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Filopodia are involved in a number of diverse cellular processes ranging from directional migration to metastatic spreading. The interplay between actin and membrane dynamics is emerging as a key factor in the formation of these structures. IRSp53 (Insulin Receptor phosphotyrosine 53kDa substrate) is the founding member of a protein family possessing a membrane curvature-inducing I-BAR domain (the so called IMD domain). In addition to the IMD, IRSp53 contains a Cdc42 and Rac interactive binding motif (CRIB) domain, an SH3 domain, a WW-binding sequence and a WH2-like domain. The SH3 domain mediates the interactions with a number of actin related proteins, among which there are VASP (Vasodilator-stimulated phosphoprotein) family proteins. VASP is a key regulator of actin structures, including filopodia and lamellipodia, exerting a variety of actin related activities whose precise regulation remains ill defined. How the IRSp53-VASP complex is regulated is unclear. In order to address this issue, we use in vitro approaches to reconstitute and characterize the IRSp53-VASP complex providing evidence that IRSp53 mediates VASP localization to PIP2 enriched membranes and enhances diverse VASP actin cytoskeletal activities. Employing fibroblasts derived from IRSp53 Knock Out mice as in vivo model systems, we also show that removal of IRSp53 impairs directional migration and invasions. This correlates with defective filopodia formation. Confocal and time-lapse microscopy analysis show that IRSp53 promotes the clustering of VASP along the plasma membrane, consistent with its role in vitro, and presumably initiating locally actin filaments elongation. Collectively these data, support a working model according to which IRSp53 drives the formation of complexes at plasma membrane site of define curvature as an initial step in filopodia formation.

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Extracellular Bacterial Pathogen Induces Host Cell Surface Reorganization to Resist Shear Stress

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Bacterial infections targeting the bloodstream lead to a wide array of devastating diseases such as septic shock and meningitis. To study this crucial type of infection, its specific environment needs to be taken into account, in particular the mechanical forces generated by the blood flow. In a previous study using Neisseria meningitidis as

a model, we observed that bacterial microcolonies forming on the endothelial cell surface in the vessel lumen are remarkably resistant to mechanical stress. The present study aims to identify the molecular basis of this resistance. N. meningitidis forms aggregates independently of host cells, yet we demonstrate here that cohesive forces involved in these bacterial aggregates are not sufficient to explain the stability of colonies on cell surfaces. Results imply that host cell attributes enhance microcolony cohesion. Microcolonies on the cell surface induce a cellular response consisting of numerous cellular protrusions similar to filopodia that come in close contact with all the bacteria in the microcolony. Consistent with a role of this cellular response, host cell lipid microdomain disruption simultaneously inhibited this response and rendered microcolonies sensitive to blood flow–generated drag forces. We then identified, by a genetic approach, the type IV pili component PilV as a triggering factor of plasma membrane reorganization, and consistently found that microcolonies formed by a pilV mutant are highly sensitive to shear stress. Our study shows that bacteria manipulate host cell functions to reorganize the host cell surface to form filopodia-like structures that enhance the cohesion of the microcolonies and therefore blood vessel colonization under the harsh conditions of the bloodstream.

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Caveolin-1 organization and trafficking is regulated by Abl kinases and mDia1

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Caveolin-1 (Cav1) biology is intimately linked to actin dynamics and adhesion receptors, but the coordinated regulation of Cav1 dynamics by actin and adhesion remains poorly understood. We describe a pathway composed of Abl tyrosine kinases and the formin mDia1 that spatially organizes an actin-linked Cav1 pool. In the absence of Abl tyrosine kinases or mDia1, or upon actin disruption, PM Cav1 forms clusters and cannot initiate inward trafficking upon loss of cell adhesion. The actin-linked Cav1 pool loses its spatial organization in the absence of actin polymerization and is dragged and clustered by depolymerizing filaments. An opposite effect is obtained by excess actin polymerization and cell stretching induced by active mDia1, which flattens caveolae, aligns Cav1 with actin fibers and rescues Cav1 trafficking defects in cells deficient for Abl kinases. In contrast, the Arp2/3-dependent actin branching activity is dispensable for Cav1 organization and inward trafficking. Notably, N-WASP regulators FBP17 and TOCA1, but not CIP4, are required for efficient Cav1 inward trafficking. Thus, the abundance of stress fibers determines the organization of Cav1 at the PM, so that deficiency of fibers leads to Cav1 clustering while an excess causes caveolae flattening. This organization is required for efficient Cav1 inward trafficking.

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V-ATPase membrane sector associates with synaptobrevin to modulate neurotransmitter release

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Acidification of synaptic vesicles by the vacuolar proton ATPase (V-ATPase) is essential for loading with neurotransmitter. We identified a direct interaction between the c-subunit of the V-ATPase membrane domain V0 and the v-SNARE VAMP2. Interaction domains were mapped to the membrane-proximal domain of VAMP2 and the cytosolic 3.4 loop of c-subunit. Acute perturbation of this interaction with c-subunit 3.4 loop peptides did not affect synaptic vesicle proton pump activity but induced a substantial decrease in release probability, inhibiting glutamatergic as well as cholinergic transmission in mammalian cortical slices and cultured sympathetic neurons respectively.

Our data identify a molecular link between V-ATPase and SNARE-mediated fusion and suggest that V-ATPase V0 sector ensures two independent functions: proton transport by a fully assembled V-ATPase and a role in SNARE-mediated neurotransmitter rerlease.

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Actin polymerization controls the organization of WASH domains at the surface of endosomes

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Sorting of cargoes in endosomes occurs through their selective enrichment into sorting platforms, where transport intermediates are generated. The WASH complex, which directly binds to lipids, activates the Arp2/3 complex and hence actin polymerization onto such sorting platforms. Here we analyzed quantitatively the role of actin polymerization in the physiology of endosomal domains containing WASH. Actin depolymerization is known to enlarge endosomes. We further show that preventing the generation of branched actin networks induces endosomal accumulation of the WASH complex, with a dramatic decrease in its recovery after photobleaching. This result suggests a built-in turnover, where the actin network, i.e. the product of the WASH complex, contributes to the dynamic cytosol/endosome exchange of the WASH complex. Moreover, impairment of actin polymerization induces lateral coalescence of WASH domains at the surface of enlarged endosomes. This behavior suggests that branched actin networks contribute to the lateral compartmentalization of endosomes into proteo-lipidic platforms.

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Membrane binding of double-C2 domain proteins measured by force spectroscopy

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Double C2-domain proteins such as synaptotagmin and DOC2 have been shown to facilitate SNARE-mediated fusion of vesicles with membranes. The exact mechanism of action of these proteins is still debated. One of the prevailing hypotheses is the ability of synaptotagmin-1 and DOC2 to lower the energy barrier for membrane fusion by inducing membrane curvature. We are using a new method that allows measurements of the force exerted by single molecules on phospholipid membranes. By comparing the force of interaction of synaptotagmin-1 and DOC2B with membranes, we hope to understand their common mechanism of action, as well as differences between the two proteins.

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Study of the Rvs protein family in the pathogenic fungus *Candida albicans*

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Candida albicans is a human commensal fungus that occurs in the gastrointestinal tract and epithelial surfaces asymptomatically. However, in immunocompromised individuals *candida* infections can even be lethal. One very characteristic feature of this yeast is its ability to switch between yeast, hyphal and pseudohyphal growth morphologies. All these morphological transitions involve a lot of membrane remodeling events. Whereas in particular the switching between yeast to hyphae has been reported to also be a crucial virulence factor.
Candida like *S. cerevisiae* utilizes endocytosis events in order to internalize receptors and cargo and to recycle parts of its membrane. In *S. cerevisiae* the proteins Rvs161 and Rvs167 are involved in the scission of the invaginated endocytic vesicles. These two proteins are part of a larger family all of which contain a BAR domain in their N-terminus. N-BARs are modules, which upon dimerization obtain a crescent shape that facilitate their ability to bind to membranes, sense and promote membrane curvature.
In *candida* the Rvs family is broader and it consists of five members. *Ca*RVS161 and *Ca*RVS167, which are involved in endocytosis, as well as *Ca*RVS167_2, *Ca*RVS162, and *Ca*NONHOM2 whose functions are currently unclear.
In an effort to elucidate the function of the Rvs protein family in *C. albicans* and to understand the role of their N-BAR domains we have employed a Yeast-2-Hybrid screen to identify the BAR-BAR interaction spectrum within this protein family. The screen has produced a number of potential interactions. Currently, these interactions are validated using *in vitro* pull-down experiments and we employ functional assays to address the membrane binding properties of these BAR domains. Also an orchestrated *in vivo* approach is employed to disentangle the function of these proteins in *candida* cells.

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Crosstalk between Arf1 and Rab1 small G proteins

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The Arf and Rab families of small G proteins are both involved in regulating membrane trafficking in eukaryotic cells. How their functions are coordinated in a particular membrane trafficking pathway are not well understood. We are studying the mechanisms by which Arf1 and Rab1 functions are linked, in particular the fact that Rab1 is involved in recruitment of the Arf1 activator GBF1 to membranes. We are studying how Rab1-mediated recruitment is integrated with other GBF1 membrane targeting mechanisms, and characterizing both the lipid and protein content of the membrane domains to which Rab1 recruits GBF1. For these analyses, we are using a number of approaches, including quantitative imaging methods (Schauer, et al., 2010), immuno- electron microscopy and development of specific lipid binding probes. We are also testing interactions between Rabs and other Arf activators to determine the generality of this Rab – Arf activation cascade.

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Synergy between membrane-curvature and liquid ordered phase-state effectively sorts lipidated proteins

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Efficient protein sorting is crucial for controlling a plethora of fundamental cellular processes. The prevailing hypothesis underlying protein transport and localization relies on the existence of nanodomains of altered fluidity, so called rafts, yet the molecular level details of this mechanism remain poorly understood (1). We recently proposed membrane curvature to be an efficient alternative mechanism for protein sorting by showing proteins anchored to membranes through amphipathic helices and lipid anchors to upconcentrate in areas of high curvature (2,3).

Here we investigated for the first time the combined effect of membrane curvature and phase state on the sorting of lipidated proteins using arrays of single liposomes (2-5). We measured the binding densities of the lipidated C-terminal NRas peptide and single palmitoylated proteins on single liposomes of different curvatures (40-800nm) as a function of lipid phase states. Our findings revealed that membranes are able to selectively recruit lipidated proteins in areas of high curvature for all phase states. This curvature selective recruitment was found to be remarkably increased for liposomes in lo phase-states as compared to the ld phases counterparts. Based on our findings we anticipate the combination of membrane curvature and a raft-like lipid phase-state to be in a highly potent mechanism for selective localization of membrane-anchored proteins (5)

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Complementary membrane curvature sensing mechanisms in the two major lipid environments of the cell

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ALPS motifs are membrane curvature sensors found in many proteins that function at the nuclear envelope and in the early secretory pathway. We have recently shown that alpha-synuclein, a causative agent of Parkinson's disease, is a second type of amphipathic helical curvature sensor that localizes to endocytic/post-Golgi vesicles (Pranke et al., 2011). Like ALPS motifs, alpha-synuclein is also an unbalanced amphipathic helix, but opposite to ALPS motifs, has a well developed polar face and a small hydrophobic face. Membranes of the ER, nuclear envelope and early Golgi are characterized by low surface charge and loosely packed phospholipids with predominantly unsaturated acyl chains, whereas TGN, endosomal and plasma membranes have high membrane packing density and membrane charge. Hence the lipid composition of the vesicles that alpha-synuclein and ALPS motifs associate with *in vitro* and in cells is complementary to each one's distinctive chemistry. These results show that complementary curvature sensing mechanisms operate in the two major lipid environments of the cell, and suggest that other alpha-synuclein-like curvature sensors might exist to regulate trafficking in the TGN-endosomal-PM membrane system. We are currently using HELIQUEST (http://heliquest.ipmc.cnrs.fr/, Gautier et al., 2008) to search for alpha-synuclein-like amphipathic helices in yeast and mammalian proteins, and will report on progress in identifying and characterizing alpha-synuclein-like curvature sensors in yeast.

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Membrane trafficking is essential for neuronal function: from growth of neurons and synapse formation to recycling of synaptic vesicles and receptors, questions concerning exocytosis and endocytosis are stimulating neurobiology research. In this study we took advantage of the transferrin receptor fused to the superecliptic pHluorin to study the two exocytosis patterns already described for the B2 adrenergic receptor and GluA1. For so called "transient" exocytosis, the receptor rapidly diffuses on the cell surface, but remains clustered for "persistent" events, and this different behaviours for single exocytosis events were analysed systematically with our model receptor expressed in cultured hippocampal neurons.

Using fast and local pH solution changes, we were able to demonstrate that a large majority of persistent events can actually be explained by a kiss&run mechanism, with a median of 2.6s for the opening time. Such experiments performed on neurons expressing B2AR-SEP or GluA1-SEP extends this mechanism to this receptors. Two colors spinning disc confocal microscopy lead us to observe the recruitment of rab35 after closure of the vesicle, in parallel to a decrease of Rab11a enrichment after fusion. Rab5a does not seem to be implicated in this recycling process, and we are currently investigating the implication of Rab4a and Rab8 in this process.

The mechanism we propose for the persistent pattern of exocytosis has important functional consequences if we consider ligand accessibility for those receptors. Colocalization experiments show that the organelles undergoing kiss&run are endosomes, the source identified previously for glutamate receptors involved in LTP, and we are characterizing those structure at the electron microscopy level.

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Dynamin 2 mutations linked to human disease impair its cellular function and highlight dynamin's role during development

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Dynamins are large GTPases implicated in membrane remodeling and cytoskeletal dynamics. Five members of the dynamin superfamily, *OPA1*, *DNM1L*, *ATL1*, *MFN2* and *DNM2*, have been previously implicated in dominant inherited diseases affecting different tissues. Heterozygous mutations in dynamin 2 (*DNM2*) have been linked to autosomal dominant Charcot-Marie Tooth peripheral neuropathy and centronuclear myopathy, two discrete progressive neuromuscular disorders, highlighting the importance of dynamin 2 for normal axonal and muscle maintenance. We report the characterization of three consanguineous patients suffering from a lethal congenital syndrome associating pleiotropic clinical symptoms. Genetic analysis identified a non-conservative point mutation in the middle domain of dynamin 2 (F379V). These findings demonstrate that impairment of dynamin 2 function leads to developmental defects in humans. Patient fibroblasts displayed reduced transferrin uptake suggesting that the F379V mutation impacts on dynamin's role in endocytosis. Expression analysis during mouse embryogenesis and the severity of the congenital syndrome observed in the patients, highlight dynamin 2's function during fetal development and that its role is conserved in vertebrates.

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The F-BAR protein NOSTRIN interacts with the FGF-R1 and is an essential component of the FGF-R signal transduction complex in endothelial cells

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F-BAR proteins are exquisitely positioned at the interface of the plasma membrane and the cytoskeleton and they play an important role in co-ordinating events involving both compartments such as endocytosis or migration. Recent reports indicate that in addition F-BAR proteins participate in growth factor signal transduction, so that the picture evolves, that F-BAR proteins might serve to integrate growth factor signals with the co-ordinated action of the plasma membrane and the cytoskeleton.

In recent years we have identified a number of interacting proteins, which assemble around the multivalent F-BAR adaptor NOSTRIN. It binds dynamin, N-WASP and endothelial nitric oxide synthase (eNOS) through its C-terminal SH3 domain and facilitates the endocytosis of eNOS. Our recent findings indicate that NOSTRIN in addition interacts with the small GTPase Rac1 and its GEF SOS and is involved in tip cell filopodia dynamics and Rac activation (see second abstract by Kovacevic et al).

With this study we aimed to further analyze the network of interacting proteins in which NOSTRIN serves as a multivalent adaptor in endothelial cells. For this purpose, we performed a yeast-two-hybrid (Y2H) screen using the C-terminal domain of human NOSTRIN as a bait and identified fibroblast growth factor-receptor 1 (FGF-R) as a novel interacting protein. We confirmed the interaction of endogenously expressed full-length proteins in endothelial cells by co-immunoprecipitation experiments. The interaction of NOSTRIN and the FGF-R1 is direct and does not dependend on NOSTRIN's SH3 domain, as demonstrated by interaction analysis using purified recombinant proteins.

Transient overexpression of NOSTRIN in HeLa cells lead to a marked increase in the level of pospho-ERK1/2 in response to FGF-2 and the knockdown of NOSTRIN by lentivirus-mediated shRNA expression reversed the effect, indicating that NOSTRIN participates in FGF-2 dependent signal transduction. Accordingly, the levels of

pospho-ERK1/2 and phospho-MEK1/2 after FGF-2 treatment were significantly decreased in mouse lung endothelial cells (MLECs) isolated from NOSTRIN KO mice in comparison to wt MLECs. Finally, the FGF-2 dependent activation of Rac in wt MLECs is abolished in NOSTRIN KO MLECs.
Taken together these data indicate, that NOSTRIN is an essential component of the FGF-R1 signalling complex and might participate in pro-angiogenic FGF-2 dependent signal transduction in endothelial cells.

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Local shaping of cortical actin by Class I myosins is required for vesicle exocytosis at the immune synapse

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Engagement of the B Cell Receptor (BCR) by surface-tethered antigens (Ag) leads to formation of a synapse that promotes Ag uptake and presentation onto MHCII molecules. Here, we highlight the membrane trafficking events and associated molecular mechanisms required for efficient Ag extraction and processing at the B cell synapse. We show that MHCII-containing lysosomes are recruited at the synapse and locally undergo exocytosis, a process that relies on the SNARE protein Vamp-7. Lysosome secretion allows the extracellular release of proteases, whose activities promote the extraction of the immobilized Ag. We further show that local re-organization of cortical actin by type I Myosins, which link the cortex to the plasma membrane, is required for lysosome exocytosis and Ag extraction. Remarkably, while the short-tail Myosin IC is recruited at the synapse and facilitates vesicle secretion and Ag uptake, the long-tail Myosin IE negatively regulates both processes. These results suggest that the two class I Myosins play antagonistic roles in the local reorganization of cortical actin for vesicle secretion and Ag uptake. The B cell synapse therefore emerges as a highly specialized site where tightly regulated exocytic and endocytic events take place thanks to the local shaping of the membrane-cytoskeleton interface by class I Myosins.

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Inhibition of phagocytosis in hiv-1 infected macrophages relies on Nef-dependent alteration of focal delivery of recycling compartments

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Phagocytosis is the mechanism used by specialised cells of the immune system such as macrophages and dendritic cells to internalise and degrade microorganisms, damaged cells or various particles. During phagocytosis, internalisation depends on cortical actin reorganisation, coordinated with a remodeling of the plasma membrane that relies on focal exocytosis of intracellular compartments at the site of particle attachment. Infection of macrophages by the Human Immunodeficiency Virus (HIV) leads to a perturbation of phagocytic function, which contributes to the increased susceptibility of the infected patients to opportunistic pathogens. The molecular basis for this phagocytic defect is not fully characterised.
Here we show that phagocytosis mediated through various receptors was inhibited in primary human macrophages infected with wild type HIV-1 but not with a *nef*-deleted virus. We observed no major perturbation of F-actin accumulation but adaptor protein 1 (AP1)-positive endosome recruitment was inhibited in HIV-1 infected cells. Expression of Nef was sufficient to inhibit phagocytosis, and myristoylation, as well as the D174-175D and L164-165L motifs involved in the association of Nef with AP complexes were important for this inhibition. Finally, we observed an alteration of the recruitment of VAMP3- and TNFα-positive recycling endosomes regulated by AP1, but not of VAMP7-positive late endosomes, in phagocytic cups of HIV-1-infected macrophages. We conclude that HIV-1 impairs optimal phagosome formation through Nef-dependent perturbation of the endosomal remodeling relying on AP1.

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EHD2 remodels caveolae membranes via specific ATP-driven targeting and oligomerisation

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EHD2 belongs to the Eps15 homology (EH) domain protein family of dynamin-related ATPases involved in membrane remodelling in the endosomal system. EHD2 dimers oligomerise into rings on highly curved membranes resulting in stimulation of the intrinsic ATPase activity. Here, we report that EHD2 is specifically and stably associated with caveolae at the plasma membrane and not involved in clathrin mediated endocytosis or endosomal recycling as previously suggested. EHD2 interacts with cavin1 and the membrane association of EHD2 is dependent on cavin1 and intact caveolae integrity. While the EH domain of EHD2 is dispensable for targeting, we identified a loop in the GTPase domain required for caveolae localisation. EHD2 overexpression promotes caveolae turnover and ATPase deficient mutants of EHD2 cause tubulation and distortion of caveolin positive membranes. Thus, following the identification of caveolin and cavins, EHD2 constitutes a third structural component of caveolae involved in the shaping of this organelle.

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The F-BAR protein NOSTRIN is necessary for proper vascular development in zebrafish and mice

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To elucidate the function of the F-BAR protein NOSTRIN *in vivo* we carried out a morpholino-mediated knockdown (KD) of NOSTRIN in developing zebrafish embryos. The NOSTRIN KD resulted in vascular defects characterized e.g. by the irregular appearance of intersegmental vessles (ISVs) with wrong connections formed between neighbouring ISVs in both morphants, misshaped dorsal londitudinal anastomotic vessels (DLAV), hindbrain edema and cranial haemorraghing. The specificity of the vascular defects was demonstrated by a dose-dependent rescue of the phenotype by re-introduction of NOSTRIN by simultaneous injection of NOSTRIN mRNA together with the SH3 splice MO.

In vivo time-lapse microscopy of NOSTRIN morphants revealed delayed occurrence of ISV sprouts and their failure to grow dorsally in a directed fashion. This was characterized by leading cells, which remained at the level of the myoseptum, explored the surrounding area and established wrong connections with the neighbouring ISV. This was associated with a change of tip cell morphology from the characteristic elongated shape in control embryos to a stub like appearance in NOSTRIN morphants and a significant reduction of the length of tip cell filopodia. In addition we generated NOSTRIN knockout mice and studied the postnatal retinal angiogenesis. Confirming our findings in zebrafish, we found a significant reduction of the mean vascular radius in NOSTRIN KO mice at postnatal day 5 (P5) and P7. This was accompanied by a decrease of the number tip cells and filopodia.

Filopodia formation is critically controlled by the activity of small GTPases of the Rho family. Here we show for the first time that NOSTRIN interacts with Rac1 (via the NOSTRIN HR1 motif) and the Rac GEF SOS (via the NOSTRIN SH3 domain and the proline-rich domain of SOS). NOSTRIN overexpression resulted in Rac1 activation, in contrast to NOSTRIN mutants unable to interact with either Rac1 or SOS. Finally, we could stimulate Rac activity in primary endothelial cells isolated from wt mice but not in cells from NOSTRIN knockout mice, confirming our previous findings that NOSTRIN is favourable for the activation of Rac. In this study we show for the first time that NOSTRIN is necessary for the co-ordinated vascular development in zebrafish and involved in postnatal retinal angiogenesis in mice. Taken together our results indicate a hitherto unknown important function of NOSTRIN for Rac1 activity during vascular development.

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Lysophosphatidate acyl transferase (LPAAT) enzymes regulate Golgi trafficking

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Membrane transport from a donor compartment to an acceptor compartment is a process that occurs through a series of events. However the first step common to all pathways is the generation of curvature in a flat membranous surface, such as the plasma membrane or a Golgi membrane, in order to form a bud. Membrane bending can be achieved by proteins that induce positive membrane curvature, and/or through local changes in lipid composition and geometry. While several membrane-curving proteins have been identified, relatively little is known about the lipid metabolising enzymes involved in membrane remodeling. Among their possible products, the closely metabolically interrelated lipids lysophospholipids (LPLs), phosphatidic acid (PA), and diacylglycerol (DAG) have intrinsic curvature and have been proposed to mediate rapid lipid geometry changes. The former might act by facilitating tubulation when in the cytosolic leaflet through its wedge like shape while the latter two lipids might destabilize tubules through their conical shape. Phospholipase A_2 (PLA_2) activity, which generates LPLs, is involved in endocytic recycling, Golgi retrograde trafficking, and Golgi structure by altering membrane tubule formation Pharmacological studies have suggested that also LPL acyltransferases (LPAATs), wich catalise the opposite reaction, play a role in membrane trafficking by catalysing the transfer of a fatty acid from an acyl-CoA donor to LPLs to generate PA
We have previously reported that BARS (Brefeldin A ADP-ribosilation substrate), a member of the CtBP (C-terminal binding protein) protein family, is able to stimulate basolaterally-directed post-Golgi carriers formation and also fission on isolated Golgi membranes by acting as an LPAAT enzyme. Later work, however, showed that this activity is not intrinsic to BARS, but it is due to a tightly associated protein. Indeed, recombinant BARS is devoid of LPAAT activity when prepared from bacterial strains genetically depleted of this enzyme. We thus reasoned that BARS could interact with an LPAAT in mammalian cells
Here we report that indeed BARS binds two Golgi localised LPAAT isoforms, LPAAT3 and LPAAT4.By using COS7 cells expressing the temperature-sensitive mutant of VSVG as a cargo reporter we show that the inhibition of LPAAT3 by either the injection of an anti-LPAAT3 antibody or the expression of a catalytically inactive LPAAT3 point mutant inhibit the formation of basolaterally-directed post-Golgi carriers. In addition, also the the use of CI976, a general LPAAT inhibitor, gives the same results indicating the involvement of PA produced by LPAAT3 in the regulation of Golgi trafficking. The same approach was used to block the activity of LPAAT4 and very similar results were obtained. At the moment we are defining the functional role of this interaction in the context of post-Golgi carrier formation.
Collectively these results led to the conclusion that the PA produced by LPAAT3 and LPAAT4 is important for membrane remodeling wich is required for Golgi trafficking.

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Molecular mechanism of membrane tethering and fusion reconstituted in vitro

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The dynamics of membrane trafficking are essential for the survival of eukaryotic cells. The molecular mechanisms of vesicle formation and consumption are highly conserved and a similar set of proteins orchestrate fusion of membranes at almost every organelle inside the cell. Rab GTPases and their effectors are responsible for vesicle tethering, while SNAREs and related regulatory proteins contribute to membrane fusion. The process of SNARE-mediated membrane fusion has been widely discussed and modelled. Less developed is the concept of vesicle tethering: many proteins are defined as tethering factors solely based on structural predictions, and a model of the transition from membrane tethering to fusion is missing. Using a cell-free approach and imaging techniques we investigated the tethering abilities and molecular requirements of proteins that are effectors of the Rab5 GTPase. Based on our results, some proteins were confirmed as tethering factors, others were not, and we discovered unexpected tethering abilities in at least one. Knowing which proteins are tethering factors, we now plan to test how they interact with SNAREs to drive membrane fusion. Our research aims to help building a model that explains how the transition from membrane tethering to fusion occurs at the molecular level.

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Pinkbar is an epithelial-specific BAR domain protein that generates planar membrane structures

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Bin/Amphipysin/Rvs (BAR) domain proteins sculpt cellular membranes, and play key roles in processes such as endocytosis, cell motility and morphogenesis. BAR domains are divided into three subfamilies: BAR and F-BAR domain proteins generate positive membrane curvature and stabilize cellular invaginations, whereas I-BAR domain proteins induce negative curvature and stabilize protrusions. We show that a previously uncharacterized member of the I-BAR subfamily, Pinkbar, is specifically expressed in intestinal epithelial cells where it localizes to Rab13-positive vesicles and to the plasma membrane
at intercellular junctions. Remarkably, the BAR domain of Pinkbar does not induce membrane tubulation, but promotes the formation of planar membrane sheets. Structural and mutagenesis analyses reveal that the BAR domain of Pinkbar has a relatively flat lipid-binding interface and that it assembles into sheet-like oligomers in crystals and in solution, which may explain its unique membrane-deforming activity.

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Cytoskeletal modulation of lipid-lipid interactions in the plasma membrane

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The cytoskeleton is suggested to be critical for forming cholesterol-dependent rafts. However, whether actin filaments are able to order membrane lipids in a manner necessary to form rafts has not been shown. To address this question, we assessed the effect of the cell cytoskeleton on the nanoscopic clustering of fluorescent lipid reporters in the plasma membrane. Specifically, we measured the fluorescence resonance energy transfer (FRET) between dialkyl carbocyanine dyes with a high affinity for ordered lipids that constitute rafts. Co-clustering of the lipophilic raft probes was sensitive to the membrane cholesterol content, the integrity of the actin cytoskeleton, and the bioavailability of phosphatidylinositol 4,5-bisphosphate. The effect was specific, since co-clustering of probes restricted to disordered lipids was not affected by these conditions. Interestingly, either depolymerizing actin filaments by treating cells with latrunculin B, or relieving cytoskeleton tension by inhibiting myosin II using blebbistatin, disrupted probe clustering regardless of affinity for raft or nonraft lipids.
Conditions that disrupted lipid ordering also altered the phosphoregulation of the Src family kinase (SFK) Lck. These data show that the cytoskeleton globally modulates lipid associations in the plasma membrane, and this property is important for SFK regulation.

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Regulation of organelle size and number by TOR kinase

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Many organelles exist in an equilibrium of fragmentation into smaller units and fusion into larger structures, which is coordinated with cell division, the increase in cell mass, and environmental conditions. The mechanism of organelle fragmentation and its coordination with the environment and cellular physiology are hardly explored. The conserved Serine/Threonine kinase TOR (target of rapamycin) is a nutrient sensor and regulates cell growth and metabolism. Out of the two TOR proteins in yeast, TOR1 and TOR2, only TOR1 is rapamycin-sensitive. TOR has only rarely been implicated in organelle homeostasis. TOR1 localizes to the vacuole membrane [1]. Recent findings in our laboratory show that TOR1 positively regulates vacuole fragmentation, suggesting that the fragmentation machinery should contain TOR targets.

We analyzed the rapamycin-sensitive phosphorylation of vacuole-associated proteins using an *in vitro* fragmentation assay that was recently developed in our group. This cell-free system reflects many properties of vacuole fission *in vivo* [2]. Protein extracts from isolated vacuoles obtained under fragmentation-promoting or -inhibiting conditions (e.g. with or without rapamycin) were analyzed by mass spectrometry (MS) for TOR-dependent changes in the phosphorylation patterns. A total of 2260 phosphosites were detected in the samples, corresponding to 1618 unique phosphopeptides on 349 individual proteins. These proteins were categorized into functional groups (e.g. fusion/fission factors, membrane transporters, membrane lipid metabolism etc.). About 2/3 of the proteins could be assigned to vacuole- or membrane-related functions, yielding a robust coverage of the vacuolar proteome.
Among the phosphorylated proteins, about 30% were found to be at least 4-fold up-or down-regulated in a TOR-dependent fashion. The enrichment of vacuolar material enabled us to detect phospho-changes more efficiently, yielding many more TOR-dependent phosphopeptides than identified in previous whole-cell studies.

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Visualisation of single endocytic vesicle formation and control of cytosol composition by simultaneous imaging and patch clamp recordings in live cells

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The formation of clathrin coated vesicles (CCVs) is a fundamental process of all eukaryotic cells which is controlled by a large number of cytosolic factors. To apprehend the spatio-temporal organisation of the tens of proteins involved in vesicle formation, an assay has been developed to detect the formation of single endocytic vesicles in live cells with a time resolution of 2 seconds (Merrifield et al. 2005; Taylor et al. 2011). Briefly, NIH 3T3 cells are transfected with - 113 -ransferring receptor fused with super-ecliptic phluorin (TfR-SEP), and cells are observed by time lapse TIRF microscopy synchronised with alternate pH changes between 7.4 and 5.5. At this latter value, TfR-SEP receptors at the membrane are not fluorescent and CCVs are observed in isolation. With this protocol, termed pulsed pH (ppH), endocytic activity is assessed by the appearance of “endocytic events” which occur at random locations on the adherent surface of cells. The frequency of these events is constant for at least 20 minutes under this protocol. This and other control experiments show the apparent innocuity of this protocol, and its possible use to assess endocytic activity at the cellular level.
The dynamics of CCV formation is controlled by a number of fundamental parameters, such as calcium or nucleotide concentration in the cytosol. To get access to these parameters, we have combined patch clamp recordings with the ppH protocol. For every cell recorded, we first performed an internal control by recording the endocytic event frequency in “cell attached” mode, and then made “whole cell” recordings with internal solutions of various compositions. A “control” solution with buffered calcium (free-calcium concentration ~20 nM), ATP and GTP permits the recording of an endocytic activity with minimal run-down. On the contrary, omission of nucleotides, or use of a non hydrolysable form of GTP, namely GTPγS, induced a block of CCV formation within minutes. This technique will allow direct determination of the parameters influencing endocytosis in non-excitable cells, and the dialysis into cells of factors which, by competing with endogenous proteins, will affect the dynamics of CCV formation.

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BRAP1 is a membrane-sculpting N-BAR domain containing protein that regulates podosome formation and phagocytosis in leukocytes

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Bin2 (Bridging integrator-2) also called BRAP1 (Breast cancer associated protein 1) is a BAR domain-containing protein with unknown function. This cytosolic protein is expressed predominantly in hematopoietic cells and highly expressed in leukemic cells and it is structurally dissimilar to other members of the BAR adaptor family (Ge and Prendegast, 2000). Interestingly, BRAP1 lacks sequences found in other BAR domain proteins to interact with c-Myc or with clathrin and the endocytotic machinery. BRAP1 also lacks a C-terminal SH3 domain found in other mammalian BAR homologous proteins such as Amphiphysin, Bin1 or Endophilin. Instead, BRAP1 includes a unique long C-terminal extension enriched in acidic amino acids and prolines. In order to understand its function, we have carried out a multidisciplinary approach that includes structural, biochemical, and cell biological studies.

We have solved the structure of its BAR domain by X-ray crystallography and we have studied its membrane binding properties in vitro and in vivo. We have found out that BRAP1 belongs to the N-BAR family of proteins. We have also studied the *in vivo* localization of BRAP1. Endogenous BRAP1 localizes at podosomes in B-cells, macrophages and basophilic cells and at the phagocytic cup of macrophages.

Podosomes are cellular structures that establish close contact with the extracellular matrix (ECM) and are highly dynamic. They are implicated in adhesion, cell migration, matrix degradation and invasion and they are found constitutively in motile nontransformed cells such as leukocytes, macrophages and osteoclasts. Structurally, they display a two-part architecture: a core of F-actin (which is perpendicular to the cell surface, surrounds a central membrane invagination and contains actin-associated proteins or membrane sculpting proteins like dynamin2) encircled by a larger ring-like structure containing focal adhesion proteins such as actinin, paxilin, talin and vinculin, cytoskeletal proteins such as myosin II and tropomyosin and other proteins like PIX, PAK and Git proteins. Members of the PAK family are major efectors of the Rho GTPases CDC42 and Rac (reviewed in Jaffer and Chernoff, 2002) and it has been shown that PAK induces reorganization of the actin cytoskeleton. It has been proposed that the PAK-PIX-Git complex would form an interface between adhesion-associated proteins of the ring and the regulation of actin dynamics in the core (Linder and Aepfelbacher, 2003; Linder and Kopp, 2005; Calle et al., 2006; Linder, 2007; Block et al., 2008).

We have found out that endogenous and transiently expressed BRAP1 localizes at the ring-like structure around the actin core. We have also demonstrated that an intact N-BAR domain is necessary and sufficient for BRAP1 targeting to podosomes and that the C-terminal unstructured tail permits BRAP1 interact with the protein alpha-PIX.

Given that BRAP1 binds PIX protein, which is, together with PAK, regulator of podosome function, we wondered if BRAP1 was also implicated in this issue. To assess the potential role of BRAP1 on podosomes, siRNA-silencing techniques were used in RBL·2H3 basophilic cells to evaluate the consequences of BRAP1 depletion on podosome´s properties. BRAP1-silenced cells show a reduced density of podosome and podosomes are not dynamic. We were able to rescue the WT phenotype by over-expressing a siRNA-insensitive construct, suggesting that the observed phenotype is a direct consequence of BRAP1 depletion and that BRAP1 possesses a modulatory effect on podosomal F-actin content. Thus, our data would suggest that BRAP1 is a new member of the PAK-PIX-Git complex that also participates on regulation of podosome formation. Further investigations need to be carried out to understand the interplay of these proteins for podosome regulation and the molecular mechanism.

Considering that BRAP1 is located at the phagocytic cup of macrophages, we have also studied its function in phagocytosis. We have found that the silencing of BRAP1 in alveolar macrophages increases phagocytosis. The opposite effect is found when overexpressing BRAP1 full-length and this effect is even more drastic when overexpressing BRAP1 N-BAR domain. All these data would suggest the implication of BRAP1 in phagocytosis.

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Extracellular bacterial pathogen Neisseria meningitidis induces host cell surface reorganization by acting directly on the lipid membrane

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Neisseria meningitidis (Nm) is a bacterium, which colonizes human vessels to cause fatal sepsis and meningitis. The general objective of this work is to understand how Nm resists forces generated by flowing blood. In a recent publication we showed that a bacteria-induced host cell membrane deformation is crucial for endothelium colonization. Bacterial aggregates proliferating on the cellular surface induce the formation of a network of filopodia that insert in between bacteria and enhances the cohesion of the bacterial aggregate. Our recent results based on chemical inhibitors suggest that Nm might act directly on the lipid bilayer in the plasma membrane to induce membrane deformation, without help of any intracellular signaling pathway or cytoskeletal structure. Two experimental models of cellular surface membranes lacking cellular content are currently being set up to test this hypothesis: (i) synthetic lipid bilayer (Giant Unilameler Vesicles or GUV) and (ii) Vesicles derived from endothelial cells obtained by stimulating bleb formation (plasma membrane sphere or PMS). Bacterial aggregates are places in contact with this vesicles and effect on lipid curvature is observed.

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Molecular dynamics simulation of the MIM I-BAR domain interactions with PIP_2-reach lipid bilayers

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The actin cytoskeleton is a highly dynamic structure that is required for various cell biological processes including morphogenesis, cell motility, endocytosis, and phagocytosis. It is regulated by a large number of actin-binding proteins. Recently discovered large multi-domain proteins MIM and IRSp53 regulate actin filament assembly and promote filopodia formation. Although the general functions of MIM's and IRSp53's individual domains are recently being uncovered, the exact mechanism by which the I-BAR domains of these proteins tubulate the PI(4,5)P2-rich membranes is largely unknown. We studied these interactions using a coarse-grained molecular dynamic simulations (total time of simulations was 500 ns). The simulations were performed for two systems with different orientation of the I-BAR domains against the membrane. We have demonstrated that after 100 ns simulation the I-BAR domain readily bind to a synthetic membrane, containing 20% PI(4,5)P2 PO lipids and 80% POPC lipids. After 500 ns of simulation the I-BAR dimer oriented by its N-termini towards the PI(4,5)P2-rich membrane and induced a local negative curvature on the membrane, thereby supporting the previously suggested hypotheses (Saarikangas et al, 2009; Zhao et al, 2010). However, we did not observe the penetration of the N-termini inside the lipid bilayer, as it was suggested previously. Neither we observed the penetration of an isolated N-terminus (the last 50 aa) inside the membrane during the 500 ns of simulation. The obtained results suggest that a local instability of the lipids may be involved into the N-terminus penetration, and that the oligomerization of I-BAR dimers may be needed to produce a stable curvature in the PI(4,5)P2-rich membrane.

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Coordination of cytskeleton and membrane dynamics in cytokinesis by PCH proteins

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Cytokinesis is a fundamental cellular process significant for almost all organisms. In eukaryotes, cytokinesis is accomplished through contraction of the contractile actomyosin ring (CAR) and intracellular membrane trafficking. To ensure that the genomes are equally partitioned between two daughter cells, these two processes need to be coordinated in a temporally and spatially regulated manner. Distorted coordination of these processes

frequently results in unequal distribution of chromosomes in daughter cells and may lead to cancer or genetic diseases. However, molecular mechanisms of coordinating CAR and membrane dynamics are poorly understood. PCH (pombe cdc15 homology) proteins are conserved adaptor proteins involved in various membrane trafficking events such as cell adhesion, cell motility and endocytosis. PCH proteins typically contain N-terminal lipid binding F-BAR domain and, in many cases, C-terminal SH3 domain(s). Structural studies showed that F-BAR domains dimerize to form intrinsic "banana-shaped" structures and bind to membranes via their positively charged concave surface to induce membrane curvature. In addition to their lipid binding affinity, many PCH proteins also bind to regulators of actin polymerization such as WASP and formins. This unique binding profile of PCH proteins prompted us to consider them as potential molecular linkers coupling actin and membrane dynamics during cytokinesis. Indeed, fungus PCH proteins, *S.pombe* cdc15p and *S.cerevisiae* Hof1, are both required for cytokinesis, but their function in animal cytokinesis remained to be elucidated. In this study, we analyzed function of PCH proteins in animal cytokinesis using fruit fly *Drosophila melanogaster* as a model organism.

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Study of Myosin 1b in a model membrane system

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Myosin 1b (Myo1b), a vertebrate myosin-I isoform, is a single-headed molecular motor using the energy of ATP hydrolysis to generate force along actin filaments. Like other myosins I, Myo1b simultaneously binds to an actin filament and to a membrane through its head domain and tail domain, respectively. Interestingly, it has been shown that Myo1b acts as a force sensor responding to a resisting force. These properties may enable Myo1b to control the dynamic changes of the morphology of cell organelles. Recently, we have demonstrated that Myo1b regulates the actin-dependent post-Golgi traffic of cargos, and generates force that controls the assembly of F-actin foci and promotes with the actin cytoskeleton the formation of tubules at the trans-Golgi network [1]. To further elucidate the mechanism by which Myo1b controls the membrane tubule formation, we develop an *in vitro* model membrane system composed of Myo1b, giant unilamellar vesicles (GUVs), and F-actin. Myo1b is successfully purified and its motor activity is confirmed by *in vitro* F-actin gliding assay. We then demonstrate that the purified Myo1b binds to GUVs containing phosphatidylinositol -4,5- biphosphate (PIP_2) by direct observation by fluorescence microscopy. Additionally, Cryo-EM observations provide the first direct evidence at molecular resolution of the binding of Myo1b to natural lipid bilayers obtained from cell extract. Finally, we study the dynamic morphological properties of GUVs in the presence of F-actin and Myo1b.

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LIST OF PARTICIPANTS

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