Year of publication 2017

Ronan Crépin, David Gentien, Angeline Duché, Audrey Rapinat, Cécile Reyes, Fariba Némati, Gérald Massonnet, Didier Decaudin, Selma Djender, Sandrine Moutel, Klervi Desrumeaux, Nathalie Cassoux, Sophie Piperno-Neumann, Sébastien Amigorena, Franck Perez, Sergio Roman Roman, Ario de Marco (2017 Feb 1)

**Nanobodies against surface biomarkers enable the analysis of tumor genetic heterogeneity in uveal melanoma Patient Derived Xenografts.**

*Pigment cell & melanoma research*: [DOI: 10.1111/pcmr.12577](https://doi.org/10.1111/pcmr.12577)

**Summary**

Monoclonal antibodies specific for biomarkers expressed on the surface of uveal melanoma (UM) cells would simplify the immune-capture and genomic characterization of heterogeneous tumor cells originated from patient derived xenografts (PDXs). Antibodies against four independent tumor antigens were isolated by panning a nanobody synthetic library. Such antibodies enabled flow-cytometry-based sorting of distinct cell sub-populations from UM PDXs and to analyze their genomic features. The complexity and specificity of the biochemical and genomic biomarker combinations mirrored the UM tumor polyclonality. The data showed that MUC18 is highly and universally displayed at the surface of UM cells with different genetic background and consequently represents a reliable pan-biomarker for their identification and purification. In contrast, the other three biomarkers were detected in very variable combinations in UM PDX cells. The availability of the identified nanobodies will be instrumental in developing clone-specific drug evaluation and rational clinical strategies based on accurate genomic profiling. This article is protected by copyright. All rights reserved.

Year of publication 2016

Hélène de Forges, Antoine Pilon, Isabelle Cantaloube, Antoine Pallandre, Anne-Marie Haghiri-Gosnet, Franck Perez, Christian Poüs (2016 Dec 6)

**Localized Mechanical Stress Promotes Microtubule Rescue.**


**Summary**

Microtubule dynamics rely on the properties of tubulin and are regulated by microtubule-associated proteins. GTP-tubulin assembles into hollow polymers, which can depolymerize upon GTP hydrolysis. Depolymerizing microtubules may stop shrinking and resume growth. Such rescues are regulated by microtubule-associated proteins like CLIP-170 and the CLASPs [1, 2]. Microtubule domains prone to rescues contain discrete regions (previously termed “GTP islands”) that retain a GTP-tubulin-like conformation in the main body of the microtubule [3]. However, the exact nature of these domains and the mechanisms controlling their occurrence and distribution are largely unknown. Here we show that collisions between growing microtubules and mechanical obstacles (including other
microtubules) in vitro result in the higher abundance of GTP-like islands in stressed microtubule regions. Furthermore, these islands were found to be efficiently generated by both lateral contacts and mechanical constraints applied to the main body of the microtubules. They were also particularly prominent where shifts in the number of protofilaments occur in the microtubule lattice. GTP-like islands and rescues frequently co-occurred at microtubule intersections in vitro and in living cells, both in crossing and in crossed microtubules. We also observed that CLIP-170 recognizes GTP-like islands in vivo and is retained at microtubule crossings. Therefore, we propose that rescues occur via a two-stage mechanism: (1) lattice defects determine potential rescue-promoting islands in the microtubule structure, and (2) CLIP-170 detects these islands to stimulate microtubule rescue. Our results reveal the interplay between rescue-promoting factors and microtubule architecture and organization to control microtubule dynamics.

Sandra Scharaw, Murat Iskar, Alessandro Ori, Gaelle Boncompain, Vibor Laketa, Ina Poser, Emma Lundberg, Franck Perez, Martin Beck, Peer Bork, Rainer Pepperkok (2016 Nov 23)

The endosomal transcriptional regulator RNF11 integrates degradation and transport of EGFR.

The Journal of cell biology : 543-558

Summary

Stimulation of cells with epidermal growth factor (EGF) induces internalization and partial degradation of the EGF receptor (EGFR) by the endo-lysosomal pathway. For continuous cell functioning, EGFR plasma membrane levels are maintained by transporting newly synthesized EGFRs to the cell surface. The regulation of this process is largely unknown. In this study, we find that EGF stimulation specifically increases the transport efficiency of newly synthesized EGFRs from the endoplasmic reticulum to the plasma membrane. This coincides with an up-regulation of the inner coat protein complex II (COPII) components SEC23B, SEC24B, and SEC24D, which we show to be specifically required for EGFR transport. Up-regulation of these COPII components requires the transcriptional regulator RNF11, which localizes to early endosomes and appears additionally in the cell nucleus upon continuous EGF stimulation. Collectively, our work identifies a new regulatory mechanism that integrates the degradation and transport of EGFR in order to maintain its physiological levels at the plasma membrane.


NaLi-H1: A universal synthetic library of humanized nanobodies providing highly functional antibodies and intrabodies.

eLife : DOI : 10.7554/eLife.16228

Summary

In vitro selection of antibodies allows to obtain highly functional binders, rapidly and at lower
cost. Here, we describe the first fully synthetic phage display library of humanized llama single domain antibody (NaLi-H1: Nanobody Library Humanized 1). Based on a humanized synthetic single domain antibody (hs2dAb) scaffold optimized for intracellular stability, the highly diverse library provides high affinity binders without animal immunization. NaLi-H1 was screened following several selection schemes against various targets (Fluorescent proteins, actin, tubulin, p53, HP1). Conformation antibodies against active RHO GTPase were also obtained. Selected hs2dAb were used in various immunoassays and were often found to be functional intrabodies, enabling tracking or inhibition of endogenous targets. Functionalization of intrabodies allowed specific protein knockdown in living cells. Finally, direct selection against the surface of tumor cells produced hs2dAb directed against tumor-specific antigens further highlighting the potential use of this library for therapeutic applications.


Summary

The Golgi apparatus is responsible for processing and sorting of secretory cargos. Microtubules are known to accelerate the transport of proteins from the endoplasmic reticulum to the Golgi apparatus and from the Golgi to the plasma membrane. However, whether post-Golgi transport strictly requires microtubules is still unclear. Using the retention using selective hooks (RUSH) system to synchronize the trafficking of cargos, we show that anterograde transport of tumor necrosis factor (TNF) is strongly reduced without microtubules. We show that two populations of Golgi elements co-exist in these cells. A centrally located and giantin-positive Golgi complex sustains trafficking while newly formed peripheral Golgi mini-stacks accumulate cargos in cells without microtubules. Using a genome-edited GFP-giantin cell line, we observe that the trafficking-competent Golgi population corresponds to the pre-existing one that was present before removal of microtubules. All Golgi elements support trafficking after long-term microtubules depletion or after relocation of Golgi proteins in the endoplasmic reticulum using Brefeldin A. Our results demonstrate that functional maturation of Golgi elements is needed to ensure post-Golgi trafficking and that microtubule-driven post-Golgi transport is not strictly required.


Summary

Systems that allow the control of protein traffic between subcellular compartments have
been valuable in elucidating trafficking mechanisms. Most current approaches rely on ligand or light-controlled dimerization, which results in either retardation or enhancement of the transport of a reporter. We developed an alternative approach for trafficking regulation that we term “controlled unmasking of targeting elements” (CUTE). Regulated trafficking is achieved by reversible masking of the signal that directs the reporter to its target organelle, relying on the streptavidin-biotin system. The targeting signal is generated within or immediately after a 38-amino acid streptavidin-binding peptide (SBP) that is appended to the reporter. The binding of coexpressed streptavidin to SBP causes signal masking, whereas addition of biotin causes complex dissociation and triggers protein transport to the target organelle. We demonstrate the application of this approach to the control of nuclear and peroxisomal protein import and the generation of biotin-dependent trafficking through the endocytic and COPI systems. By simultaneous masking of COPI and endocytic signals, we were able to generate a synthetic pathway for efficient transport of a reporter from the plasma membrane to the endoplasmic reticulum.

Year of publication 2015

Frédéric Canal, Elodie Anthony, Aurianne Lescure, Elaine Del Nery, Jacques Camonis, Franck Perez, Bruno Ragazzon, Christine Perret (2015 Dec 31)
A kinome siRNA screen identifies HGS as a potential target for liver cancers with oncogenic mutations in CTNNB1.

Summary

Aberrant activation of the Wnt/β-catenin pathway is a major and frequent event in liver cancer, but inhibition of oncogenic β-catenin signaling has proven challenging. The identification of genes that are synthetically lethal in β-catenin-activated cancer cells would provide new targets for therapeutic drug design.

N W Andrews, F Perez (2015 Dec 15)
The plasma membrane repair shop: Fixing the damage.
Seminars in cell & developmental biology : 1 : DOI : 10.1016/j.semcdb.2015.11.008

Summary

José I Valenzuela, Franck Perez (2015 Oct 27)
Diversifying the secretory routes in neurons.
Frontiers in neuroscience : 358 : DOI : 10.3389/fnins.2015.00358

Summary

Nervous system homeostasis and synaptic function need dedicated mechanisms to locally
regulate the molecular composition of the neuronal plasma membrane and allow the development, maintenance and plastic modification of the neuronal morphology. The cytoskeleton and intracellular trafficking lies at the core of all these processes. In most mammalian cells, the Golgi apparatus (GA) is at the center of the biosynthetic pathway, located in the proximity of the microtubule-organizing center. In addition to this central localization, the somatic GA in neurons is complemented by satellite Golgi outposts (GOPs) in dendrites, which are essential for dendritic morphogenesis and are emerging like local stations of membranes trafficking to synapses. Largely, GOPs participation in post-ER trafficking has been determined by imaging the transport of the exogenous protein VSVG. Here we review the diversity of neuronal cargoes that traffic through GOPs and the assortment of different biosynthetic routes to synapses. We also analyze the recent advances in understanding the role of cytoskeleton and Golgi matrix proteins in the biogenesis of GOPs and how the diversity of secretory routes can be generated.

Ana Joaquina Jimenez, Franck Perez (2015 Oct 24)
Physico-chemical and biological considerations for membrane wound evolution and repair in animal cells.
Seminars in cell & developmental biology : 2-9 : DOI : 10.1016/j.semcdb.2015.09.023

Summary

Membrane damage is a daily threat to the life of a cell, especially cells from muscles, gut, epidermis and vasculature, tissues that are particularly subjected to mechanical stress. Damages can come from different sources and give rise to different holes in terms of size and nature. For example, while some holes are simply scratches in the lipid bilayer, others are delimited by pore forming proteins. It is thus expectable that these wounds will not evolve similarly in a cellular context, and that repair mechanisms will differ to a certain extent. It would therefore be misleading to fully generalize cell membrane damage and repair, and consider it as one universal phenomenon. Indeed, damage has been observed in cells ranging from the rather small mammalian cells (∼30μm) to the very big Urchin egg (∼100μm). Moreover, the wounds observed or artificially induced in eukaryotic cells range from some nanometers to several micrometers, and can be delimited by particular molecules as mentioned before. This chapter aims at reviewing the different physico-chemical and biological parameters that can influence wound evolution in cells and to conciliate the different repair mechanisms that have been described by evaluating them in their cellular and wound type context.

TECPR2 Cooperates with LC3C to Regulate COPII-Dependent ER Export.
Molecular cell : 89-104 : DOI : 10.1016/j.molcel.2015.09.010
Summary

Hereditary spastic paraplegias (HSPs) are a diverse group of neurodegenerative diseases that are characterized by axonopathy of the corticospinal motor neurons. A mutation in the gene encoding for Tectonin β-propeller containing protein 2 (TECPR2) causes HSP that is complicated by neurological symptoms. While TECPR2 is a human ATG8 binding protein and positive regulator of autophagy, the exact function of TECPR2 is unknown. Here, we show that TECPR2 associates with several trafficking components, among them the COPII coat protein SEC24D. TECPR2 is required for stabilization of SEC24D protein levels, maintenance of functional ER exit sites (ERES), and efficient ER export in a manner dependent on binding to lipidated LC3C. TECPR2-deficient HSP patient cells display alterations in SEC24D abundance and ER export efficiency. Additionally, TECPR2 and LC3C are required for autophagosome formation, possibly through maintaining functional ERES. Collectively, these results reveal that TECPR2 functions as molecular scaffold linking early secretion pathway and autophagy.

Takuya Terai, Moe Kohno, Gaelle Boncompain, Shigeru Sugiyama, Nae Saito, Ryo Fujikake, Tasuku Ueno, Toru Komatsu, Kenjiro Hanaoka, Takayoshi Okabe, Yasuteru Urano, Franck Perez, Tetsuo Nagano (2015 Aug 12)

Artificial Ligands of Streptavidin (ALiS): Discovery, Characterization, and Application for Reversible Control of Intracellular Protein Transport.
Journal of the American Chemical Society : 10464-7 : DOI : 10.1021/jacs.5b05672

Summary

Artificial ligands of streptavidin (ALiS) with association constants of ~10(6) M(-1) were discovered by high-throughput screening of our chemical library, and their binding characteristics, including X-ray crystal structure of the streptavidin complex, were determined. Unlike biotin and its derivatives, ALiS exhibits fast dissociation kinetics and excellent cell permeability. The streptavidin-ALiS system provides a novel, practical compound-dependent methodology for repeated reversible cycling of protein localization between intracellular organelle.


Resolving bundled microtubules using anti-tubulin nanobodies.
Nature communications : 7933 : DOI : 10.1038/ncomms8933

Summary

Microtubules are hollow biopolymers of 25-nm diameter and are key constituents of the
cytoskeleton. In neurons, microtubules are organized differently between axons and dendrites, but their precise organization in different compartments is not completely understood. Super-resolution microscopy techniques can detect specific structures at an increased resolution, but the narrow spacing between neuronal microtubules poses challenges because most existing labelling strategies increase the effective microtubule diameter by 20-40 nm and will thereby blend neighbouring microtubules into one structure. Here we develop single-chain antibody fragments (nanobodies) against tubulin to achieve super-resolution imaging of microtubules with a decreased apparent diameter. To test the resolving power of these novel probes, we generate microtubule bundles with a known spacing of 50-70 nm and successfully resolve individual microtubules. Individual bundled microtubules can also be resolved in different mammalian cells, including hippocampal neurons, allowing novel insights into fundamental mechanisms of microtubule organization in cell- and neurobiology.

Diana Molino, Sébastien Nola, Sin Man Lam, Agathe Verraes, Véronique Proux-Gillardeaux, Gaëlle Boncompain, Franck Perez, Markus Wenk, Guanghou Shui, Lydia Danglot, Thierry Galli (2015 Jul 22)

**Role of tetanus neurotoxin insensitive vesicle-associated membrane protein in membrane domains transport and homeostasis.**

*Cellular logistics*: e1025182

**Summary**

Biological membranes in eukaryotes contain a large variety of proteins and lipids often distributed in domains in plasma membrane and endomembranes. Molecular mechanisms responsible for the transport and the organization of these membrane domains along the secretory pathway still remain elusive. Here we show that vesicular SNARE TI-VAMP/VAMP7 plays a major role in membrane domains composition and transport. We found that the transport of exogenous and endogenous GPI-anchored proteins was altered in fibroblasts isolated from VAMP7-knockout mice. Furthermore, disassembly and reformation of the Golgi apparatus induced by Brefeldin A treatment and washout were impaired in VAMP7-depleted cells, suggesting that loss of VAMP7 expression alters biochemical properties and dynamics of the Golgi apparatus. In addition, lipid profiles from these knockout cells indicated a defect in glycosphingolipids homeostasis. We conclude that VAMP7 is required for effective transport of GPI-anchored proteins to cell surface and that VAMP7-dependent transport contributes to both sphingolipids and Golgi homeostasis.

Anne Chevrel, Agathe Urvoas, Ines Li de la Sierra-Gallay, Magali Aumont-Nicaise, Sandrine Moutel, Michel Desmadril, Franck Perez, Alexis Gautreau, Herman van Tilbeurgh, Philippe Minard, Marie Valerio-Lepiniec (2015 Jul 17)

**Specific GFP-binding artificial proteins (αRep): a new tool for in vitro to live cell applications.**

*Bioscience reports*: [DOI: 10.1042/BSR20150080]
Summary

A family of artificial proteins, named αRep, based on a natural family of helical repeat was previously designed. αRep members are efficiently expressed, folded and extremely stable proteins. A large αRep library was constructed creating proteins with a randomized interaction surface. In the present study, we show that the αRep library is an efficient source of tailor-made specific proteins with direct applications in biochemistry and cell biology. From this library, we selected by phage display αRep binders with nanomolar dissociation constants against the GFP. The structures of two independent αRep binders in complex with the GFP target were solved by X-ray crystallography revealing two totally different binding modes. The affinity of the selected αReps for GFP proved sufficient for practically useful applications such as pull-down experiments. αReps are disulfide free proteins and are efficiently and functionally expressed in eukaryotic cells: GFP-specific αReps are clearly sequestered by their cognate target protein addressed to various cell compartments. These results suggest that αRep proteins with tailor-made specificity can be selected and used in living cells to track, modulate or interfere with intracellular processes.