Année de publication : 2019

Lou Fourriere, Amal Kasri, Nelly Gareil, Sabine Bardin, Hugo Bousquet, David Pereira, Franck Perez, Bruno Goud, Gaelle Boncompain, Stéphanie Miserey-Lenkei (2019 May 31)

RAB6 and microtubules restrict protein secretion to focal adhesions.

The Journal of cell biology : DOI : 10.1083/jcb.201805002

Résumé

To ensure their homeostasis and sustain differentiated functions, cells continuously transport diverse cargos to various cell compartments and in particular to the cell surface. Secreted proteins are transported along intracellular routes from the endoplasmic reticulum through the Golgi complex before reaching the plasma membrane along microtubule tracks. Using a synchronized secretion assay, we report here that exocytosis does not occur randomly at the cell surface but on localized hotspots juxtaposed to focal adhesions. Although microtubules are involved, the RAB6-dependent machinery plays an essential role. We observed that, irrespective of the transported cargos, most post-Golgi carriers are positive for RAB6 and that its inactivation leads to a broad reduction of protein secretion. RAB6 may thus be a general regulator of post-Golgi secretion.

Année de publication : 2018

Guillaume Kulakowski, Hugo Bousquet Jean-Baptiste Manneville, Patricia Bassereau, Bruno Goud, Lena K. Oesterlin (2018 Apr 6)

Lipid packing defects and membrane charge control RAB GTPase recruitment.

Traffic : 19 : 536-545 : DOI : 10.1111/tra.12568

Résumé

Specific intracellular localization of RAB GTPases has been reported to be dependent on protein factors, but the contribution of the membrane physicochemical properties to this process has been poorly described. Here, we show that three RAB proteins (RAB1/RAB5/RAB6) preferentially bind in vitro to disordered and curved membranes, and that this feature is uniquely dependent on their prenyl group. Our results imply that the addition of a prenyl group confers to RAB proteins, and most probably also to other prenylated proteins, the ability to sense lipid packing defects induced by unsaturated conical-shaped lipids and curvature. Consistently, RAB recruitment increases with the amount of lipid packing defects, further indicating that these defects drive RAB membrane targeting. Membrane binding of RAB35 is also modulated by lipid packing defects but primarily dependent on negatively charged lipids. Our results suggest that a balance between hydrophobic insertion of the prenyl group into lipid packing defects and electrostatic interactions of the RAB C-terminal region with charged membranes tunes the specific intracellular localization of RAB proteins.

Jean-Marie Carpier, Andres E Zucchetti, Laurence Bataille, Stéphanie Dogniaux, Massiullah Shafaq-Zadah, Sabine Bardin, Marco Lucchino, Mathieu Maurin, Leonel D Joannas, Joao Gamelas Magalhaes, Ludger Johannes, Thierry Galli, Bruno Goud, Claire Hivroz (2018 Feb 15)
Rab6-dependent retrograde traffic of LAT controls immune synapse formation and T cell activation.
The Journal of experimental medicine : 1245-1265 : DOI : 10.1084/jem.20162042

Résumé

The adapter molecule linker for activation of T cells (LAT) orchestrates the formation of signalosomes upon T cell receptor (TCR) stimulation. LAT is present in different intracellular pools and is dynamically recruited to the immune synapse upon stimulation. However, the intracellular traffic of LAT and its function in T lymphocyte activation are ill defined. We show herein that LAT, once internalized, transits through the Golgi-trans-Golgi network (TGN), where it is repolarized to the immune synapse. This retrograde transport of LAT depends on the small GTPase Rab6 and the target soluble -ethylmaleimide-sensitive factor attachment protein receptor (t-SNARE) Syntaxin-16, two regulators of the endosome-to-Golgi/TGN retrograde transport. We also show in vitro in Syntaxin-16- or Rab6-silenced human cells and in vivo in CD4 T lymphocytes of the Rab6 knockout mouse that this retrograde traffic controls TCR stimulation. These results establish that the retrograde traffic of LAT from the plasma membrane to the Golgi-TGN controls the polarized delivery of LAT at the immune synapse and T lymphocyte activation.

Bruno Goud, Daniel Louvard (2018 Feb 1)
[Cell complexity should be placed at the heart of cancer research].
Medecine sciences : M/S : 63-71 : DOI : 10.1051/medsci/20183401015

Résumé

Genetic and most likely epigenetic alterations occurring during tumor progression and metastatic process lead to a broad deregulation of major cellular functions. However, the molecular mechanisms involved are still poorly understood. To understand them, the cell, the basic unit of life, remains more than ever the essential level to integrate the functional impact of genetics and epigenetics processes in the light of the global economy of the normal and cancerous cell, and of its interactions with its microenvironment.

Année de publication : 2017

Sara Bizzotto, Ana Uzquiano, Florent Dingli, Dmitry Ershov, Anne Houllier, Guillaume Arras, Mark Richards, Damarys Loew, Nicolas Minc, Alexandre Croquelois, Anne Houdusse, Fiona Francis (2017 Dec 13)
Eml1 loss impairs apical progenitor spindle length and soma shape in the

developing cerebral cortex.
Scientific reports : 17308 : DOI : 10.1038/s41598-017-15253-4

Résumé

The ventricular zone (VZ) of the developing cerebral cortex is a pseudostratified epithelium that contains progenitors undergoing precisely regulated divisions at its most apical side, the ventricular lining (VL). Mitotic perturbations can contribute to pathological mechanisms leading to cortical malformations. The HeCo mutant mouse exhibits subcortical band heterotopia (SBH), likely to be initiated by progenitor delamination from the VZ early during corticogenesis. The causes for this are however, currently unknown. Eml1, a microtubule (MT)-associated protein of the EMAP family, is impaired in these mice. We first show that MT dynamics are perturbed in mutant progenitor cells in vitro. These may influence interphase and mitotic MT mechanisms and indeed, centrosome and primary cilia were altered and spindles were found to be abnormally long in HeCo progenitors. Consistently, MT and spindle length regulators were identified in EML1 pulldowns from embryonic brain extracts. Finally, we found that mitotic cell shape is also abnormal in the mutant VZ. These previously unidentified VZ characteristics suggest altered cell constraints which may contribute to cell delamination.

Stéphanie Miserey-Lenkei, Hugo Bousquet, Olena Pylypenko, Sabine Bardin, Ariane Dimitrov, Gaëlle Bressanelli, Raja Bonifay, Vincent Fraisier, Catherine Guillou, Cécile Bougeret, Anne Houdusse, Arnaud Echard, Bruno Goud (2017 Nov 3)
Coupling fission and exit of RAB6 vesicles at Golgi hotspots through kinesin-myosin interactions.
Nature communications : 1254 : DOI : 10.1038/s41467-017-01266-0

Résumé

The actin and microtubule cytoskeletons play important roles in Golgi structure and function, but how they are connected remain poorly known. In this study, we investigated whether RAB6 GTPase, a Golgi-associated RAB involved in the regulation of several transport steps at the Golgi level, and two of its effectors, Myosin IIA and KIF20A participate in the coupling between actin and microtubule cytoskeleton. We have previously shown that RAB6-Myosin IIA interaction is critical for the fission of RAB6-positive transport carriers from Golgi/TGN membranes. Here we show that KIF20A is also involved in the fission process and serves to anchor RAB6 on Golgi/TGN membranes near microtubule nucleating sites. We provide evidence that the fission events occur at a limited number of hotspots sites. Our results suggest that coupling between actin and microtubule cytoskeletons driven by Myosin II and KIF20A ensures the spatial coordination between RAB6-positive vesicles fission from Golgi/TGN membranes and their exit along microtubules.

Anand Patwardhan, Sabine Bardin, Stéphanie Miserey-Lenkei, Lionel Larue, Bruno Goud, Graça Raposo, Cédric Delevoye (2017 Jun 14)

Routing of the RAB6 secretory pathway towards the lysosome related organelle of melanocytes.
Nature communications : 15835 : DOI : 10.1038/ncomms15835

Résumé

Exocytic carriers convey neo-synthesized components from the Golgi apparatus to the cell surface. While the release and anterograde movement of Golgi-derived vesicles require the small GTPase RAB6, its effector ELKS promotes the targeting and docking of secretory vesicles to particular areas of the plasma membrane. Here, we show that specialized cell types exploit and divert the secretory pathway towards lysosome related organelles. In cultured melanocytes, the secretory route relies on RAB6 and ELKS to directly transport and dock Golgi-derived carriers to melanosomes. By delivering specific cargos, such as MART-1 and TYRP2/ DCT, the RAB6/ELKS-dependent secretory pathway controls the formation and maturation of melanosomes but also pigment synthesis. In addition, pigmentation defects are observed in RAB6 KO mice. Our data together reveal for the first time that the secretory pathway can be directed towards intracellular organelles of endosomal origin to ensure their biogenesis and function.

Charlotte Alibert, Bruno Goud, Jean-Baptiste Manneville (2017 Mar 1)
Are cancer cells really softer than normal cells?
Biology of the cell : DOI : 10.1111/boc.201600078

Résumé

Solid tumors are often first diagnosed by palpation, suggesting that the tumor is more rigid than its surrounding environment. Paradoxically, individual cancer cells appear to be softer than their healthy counterparts. In this review, we first list the physiological reasons indicating that cancer cells may be more deformable than normal cells. Next, we describe the biophysical tools that have been developed in recent years to characterize and model cancer cell mechanics. By reviewing the experimental studies that compared the mechanics of individual normal and cancer cells, we argue that cancer cells can indeed be considered as softer than normal cells. We then focus on the intracellular elements that could be responsible for the softening of cancer cells. Finally, we ask whether the mechanical differences between normal and cancer cells can be used as diagnostic or prognostic markers of cancer progression. This article is protected by copyright. All rights reserved.

Année de publication : 2016

Mandal K, Asnacios A, Goud B, Manneville JB (2016 Oct 31)
Mapping intracellular mechanics on micropatterned substrates.
Proceedings of the national academy of sciences of the united states of america : pii. 201605112 : DOI : 10.1073/pnas.1609342113

Résumé

The mechanical properties of cells impact on their architecture, their migration, intracellular trafficking, and many other cellular functions and have been shown to be modified during cancer progression. We have developed an approach to map the intracellular mechanical properties of living cells by combining micropatterning and optical tweezers-based active microrheology. We optically trap micrometer-sized beads internalized in cells plated on crossbow-shaped adhesive micropatterns and track their displacement following a step displacement of the cell. The local intracellular complex shear modulus is measured from the relaxation of the bead position assuming that the intracellular microenvironment of the bead obeys power-law rheology. We also analyze the data with a standard viscoelastic model and compare with the power-law approach. We show that the shear modulus decreases from the cell center to the periphery and from the cell rear to the front along the polarity axis of the micropattern. We use a variety of inhibitors to quantify the spatial contribution of the cytoskeleton, intracellular membranes, and ATP-dependent active forces to intracellular mechanics and apply our technique to differentiate normal and cancer cells.

Olena Pylypenko, Tobias Welz, Janine Tittel, Martin Kollmar, Florian Chardon, Gilles Malherbe, Sabine Weiss, Carina Ida Luise Michel, Annette Samol-Wolf, Andreas Till Grasskamp, Alistair Hume, Bruno Goud, Bruno Baron, Patrick England, Margaret A Titus, Petra Schwille, Thomas Weidemann, Anne Houdusse, Eugen Kerkhoff (2016 Sep 14)

Coordinated recruitment of Spir actin nucleators and myosin V motors to Rab11 vesicle membranes.

eLife : DOI : 10.7554/eLife.17523

Résumé

There is growing evidence for a coupling of actin assembly and myosin motor activity in cells. However, mechanisms for recruitment of actin nucleators and motors on specific membrane compartments remain unclear. Here we report how Spir actin nucleators and myosin V motors coordinate their specific membrane recruitment. The myosin V globular tail domain (MyoV-GTD) interacts directly with an evolutionarily conserved Spir sequence motif. We determined crystal structures of MyoVa-GTD bound either to the Spir-2 motif or to Rab11 and show that a Spir-2:MyoVa:Rab11 complex can form. The ternary complex architecture explains how Rab11 vesicles support coordinated F-actin nucleation and myosin force generation for vesicle transport and tethering. New insights are also provided into how myosin activation can be coupled with the generation of actin tracks. Since MyoV binds several Rab GTPases, synchronized nucleator and motor targeting could provide a common mechanism to control force generation and motility in different cellular processes.

Jean-Baptiste Brault, Cécile Khou, Justine Basset, Laure Coquand, Vincent Fraisier, Marie-Pascale Frenkiel, Bruno Goud, Jean-Claude Manuguerra, Nathalie Pardigon, Alexandre D Baffet (2016 Jul 26)

Comparative Analysis Between Flaviviruses Reveals Specific Neural Stem Cell Tropism for Zika Virus in the Mouse Developing Neocortex.
EBioMedicine : DOI : S2352-3964(16)30323-1

Résumé

The recent Zika outbreak in South America and French Polynesia was associated with an epidemic of microcephaly, a disease characterized by a reduced size of the cerebral cortex. Other members of the Flavivirus genus, including West Nile virus (WNV), can cause encephalitis but were not demonstrated to cause microcephaly. It remains unclear whether Zika virus (ZIKV) and other flaviviruses may infect different cell populations in the developing neocortex and lead to distinct developmental defects. Here, we describe an assay to infect mouse E15 embryonic brain slices with ZIKV, WNV and dengue virus serotype 4 (DENV-4). We show that this tissue is able to support viral replication of ZIKV and WNV, but not DENV-4. Cell fate analysis reveals a remarkable tropism of ZIKV infection for neural stem cells. Closely related WNV displays a very different tropism of infection, with a bias towards neurons. We further show that ZIKV infection, but not WNV infection, impairs cell cycle progression of neural stem cells. Both viruses inhibited apoptosis at early stages of infection. This work establishes a powerful comparative approach to identify ZIKV-specific alterations in the developing neocortex and reveals specific preferential infection of neural stem cells by ZIKV.

Laura Picas, Frederique Gaits-Iacovoni, Bruno Goud (2016 Apr 20)
The emerging role of phosphoinositide clustering in intracellular trafficking and signal transduction.
F1000Research : DOI : 10.12688/f1000research.7537.1

Résumé

Phosphoinositides are master regulators of multiple cellular processes: from vesicular trafficking to signaling, cytoskeleton dynamics, and cell growth. They are synthesized by the spatiotemporal regulated activity of phosphoinositide-metabolizing enzymes. The recent observation that some protein modules are able to cluster phosphoinositides suggests that alternative or complementary mechanisms might operate to stabilize the different phosphoinositide pools within cellular compartments. Herein, we discuss the different known and potential molecular players that are prone to engage phosphoinositide clustering and elaborate on how such a mechanism might take part in the regulation of intracellular trafficking and signal transduction.

Daniel Koch, Amrita Rai, Imtiaz Ali, Nathalie Bleimling, Timon Friese, Andreas Brockmeyer, Petra Janning, Bruno Goud, Aymelt Itzen, Matthias P Müller, Roger S Goody (2016 Feb 27)
A pull-down procedure for the identification of unknown GEFs for small GTPases.
Small GTPases : 93-106 : DOI : 10.1080/21541248.2016.1156803

Résumé

Members of the family of small GTPases regulate a variety of important cellular functions. In order to accomplish this, tight temporal and spatial regulation is absolutely necessary. The two most important factors for this regulation are GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), the latter being responsible for the activation of the GTPase downstream pathways at the correct location and time. Although a large number of exchange factors have been identified, it is likely that a similarly large number remains unidentified. We have therefore developed a procedure to specifically enrich GEF proteins from biological samples making use of the high affinity binding of GEFs to nucleotide-free GTPases. In order to verify the results of these pull-down experiments, we have additionally developed two simple validation procedures: An in vitro transcription/translation system coupled with a GEF activity assay and a yeast two-hybrid screen for detection of GEFs. Although the procedures were established and tested using the Rab protein Sec4, the similar basic principle of action of all nucleotide exchange factors will allow the method to be used for identification of unknown GEFs of small GTPases in general.

Massiullah Shafaq-Zadah, Carina S Gomes-Santos, Sabine Bardin, Paolo Maiuri, Mathieu Maurin, Julian Iranzo, Alexis Gautreau, Christophe Lamaze, Patrick Caswell, Bruno Goud, Ludger Johannes (2016 Jan 8)

Persistent cell migration and adhesion rely on retrograde transport of β(1) integrin.

Nature cell biology : 54-64 : DOI : 10.1038/ncb3287

Résumé

Integrins have key functions in cell adhesion and migration. How integrins are dynamically relocalized to the leading edge in highly polarized migratory cells has remained unexplored. Here, we demonstrate that β1 integrin (known as PAT-3 in Caenorhabditis elegans), but not β3, is transported from the plasma membrane to the trans-Golgi network, to be resecreted in a polarized manner. This retrograde trafficking is restricted to the non-ligand-bound conformation of β1 integrin. Retrograde trafficking inhibition abrogates several β1-integrin-specific functions such as cell adhesion in early embryonic development of mice, and persistent cell migration in the developing posterior gonad arm of C. elegans. Our results establish a paradigm according to which retrograde trafficking, and not endosomal recycling, is the key driver for β1 integrin function in highly polarized cells. These data more generally suggest that the retrograde route is used to relocalize plasma membrane machinery from previous sites of function to the leading edge of migratory cells.

Année de publication : 2015

Jonna Alanko, Anja Mai, Guillaume Jacquemet, Kristine Schauer, Riina Kaukonen, Markku Saari, Bruno Goud, Johanna Ivaska (2015 Oct 6)

Integrin endosomal signalling suppresses anoikis.
Nature cell biology : 1412-21 : DOI : 10.1038/ncb3250

Résumé

Integrin-containing focal adhesions transmit extracellular signals across the plasma membrane to modulate cell adhesion, signalling and survival. Although integrins are known to undergo continuous endo/exocytic traffic, the potential impact of endocytic traffic on integrin-induced signals is unknown. Here, we demonstrate that integrin signalling is not restricted to cell-ECM adhesions and identify an endosomal signalling platform that supports integrin signalling away from the plasma membrane. We show that active focal adhesion kinase (FAK), an established marker of integrin-ECM downstream signalling, localizes with active integrins on endosomes. Integrin endocytosis positively regulates adhesion-induced FAK activation, which is early endosome antigen-1 and small GTPase Rab21 dependent. FAK binds directly to purified endosomes and becomes activated on them, suggesting a role for endocytosis in enhancing distinct integrin downstream signalling events. Finally, endosomal integrin signalling contributes to cancer-related processes such as anoikis resistance, anchorage independence and metastasis.