Année de publication : 2021

Valentin Laplaud, Nicolas Levernier, Judith Pineau, Mabel San Roman, Lucie Barbier, Pablo J Sáez, Ana-Maria Lennon-Duménil, Pablo Vargas, Karsten Kruse, Olivia du Roure, Matthieu Piel, Julien Heuvingh (2021 Jul 3)

Pinching the cortex of live cells reveals thickness instabilities caused by myosin II motors.

Science advances : DOI : eabe3640

Résumé

The cell cortex is a contractile actin meshwork, which determines cell shape and is essential for cell mechanics, migration, and division. Because its thickness is below optical resolution, there is a tendency to consider the cortex as a thin uniform two-dimensional layer. Using two mutually attracted magnetic beads, one inside the cell and the other in the extracellular medium, we pinch the cortex of dendritic cells and provide an accurate and time-resolved measure of its thickness. Our observations draw a new picture of the cell cortex as a highly dynamic layer, harboring large fluctuations in its third dimension because of actomyosin contractility. We propose that the cortex dynamics might be responsible for the fast shape-changing capacity of highly contractile cells that use amoeboid-like migration.

Ana Martins Figueiredo, Pedro Barbacena, Ana Russo, Silvia Vaccaro, Daniela Ramalho, Andreia Pena, Aida Pires Lima, Rita Rua Ferreira, Marta Alves Fidalgo, Fatima El-Marjou, Yulia Carvalho, Francisca Ferreira Vasconcelos, Ana-Maria Lennon-Duménil, Danijela Matic Vignjevic, Claudio Areias Franco (2021 Apr 27)

Endothelial cell invasion is controlled by dactylopodia.

Proceedings of the National Academy of Sciences of the United States of America : DOI : e2023829118

Résumé

Sprouting angiogenesis is fundamental for development and contributes to cancer, diabetic retinopathy, and cardiovascular diseases. Sprouting angiogenesis depends on the invasive properties of endothelial tip cells. However, there is very limited knowledge on how tip cells invade into tissues. Here, we show that endothelial tip cells use dactylopodia as the main cellular protrusion for invasion into nonvascular extracellular matrix. We show that dactylopodia and filopodia protrusions are balanced by myosin IIA (NMIIA) and actin-related protein 2/3 (Arp2/3) activity. Endothelial cell-autonomous ablation of NMIIA promotes excessive dactylopodia formation in detriment of filopodia. Conversely, endothelial cell-autonomous ablation of Arp2/3 prevents dactylopodia development and leads to excessive filopodia formation. We further show that NMIIA inhibits Rac1-dependent activation of Arp2/3 by regulating the maturation state of focal adhesions. Our discoveries establish a comprehensive model of how endothelial tip cells regulate its protrusive activity and will pave the way toward strategies to block invasive tip cells during sprouting angiogenesis.

Andreia Mendes, Julien P Gigan, Christian Rodriguez Rodrigues, Sébastien A Choteau, Doriane Sanseau, Daniela Barros, Catarina Almeida, Voahirana Camosseto, Lionel Chasson, Adrienne W Paton, James C Paton, Rafael J Argüello, Ana-Maria Lennon-Duménil, Evelina Gatti, Philippe Pierre (2021 Jan 14)

Proteostasis in dendritic cells is controlled by the PERK signaling axis independently of ATF4.

Life science alliance : DOI : e202000865

Résumé

In stressed cells, phosphorylation of eukaryotic initiation factor 2α (eIF2α) controls transcriptome-wide changes in mRNA translation and gene expression known as the integrated stress response. We show here that DCs are characterized by high eIF2α phosphorylation, mostly caused by the activation of the ER kinase PERK (EIF2AK3). Despite high p-eIF2α levels, DCs display active protein synthesis and no signs of a chronic integrated stress response. This biochemical specificity prevents translation arrest and expression of the transcription factor ATF4 during ER-stress induction by the subtilase cytotoxin (SubAB). PERK inactivation, increases globally protein synthesis levels and regulates IFN-β expression, while impairing LPS-stimulated DC migration. Although the loss of PERK activity does not impact DC development, the cross talk existing between actin cytoskeleton dynamics; PERK and eIF2α phosphorylation is likely important to adapt DC homeostasis to the variations imposed by the immune contexts.

Année de publication : 2020

A J Lomakin, C J Cattin, D Cuvelier, Z Alraies, M Molina, G P F Nader, N Srivastava, P J Sáez, J M Garcia-Arcos, I Y Zhitnyak, A Bhargava, M K Driscoll, E S Welf, R Fiolka, R J Petrie, N S De Silva, J M González-Granado, N Manel, A M Lennon-Duménil, D J Müller, M Piel (2020 Oct 16)

The nucleus acts as a ruler tailoring cell responses to spatial constraints.

Science (New York, N.Y.) : DOI : eaba2894

Résumé

The microscopic environment inside a metazoan organism is highly crowded. Whether individual cells can tailor their behavior to the limited space remains unclear. In this study, we found that cells measure the degree of spatial confinement by using their largest and stiffest organelle, the nucleus. Cell confinement below a resting nucleus size deforms the nucleus, which expands and stretches its envelope. This activates signaling to the actomyosin cortex via nuclear envelope stretch-sensitive proteins, up-regulating cell contractility. We established that the tailored contractile response constitutes a nuclear ruler-based signaling pathway involved in migratory cell behaviors. Cells rely on the nuclear ruler to modulate the motive force that enables their passage through restrictive pores in complex three-dimensional environments, a process relevant to cancer cell invasion, immune responses, and embryonic development.

Aleksandra S Chikina, Francesca Nadalin, Mathieu Maurin, Mabel San-Roman, Thibault Thomas-Bonafos, Xin V Li, Sonia Lameiras, Sylvain Baulande, Sandrine Henri, Bernard Malissen, Livia Lacerda Mariano, Jorge Barbazan, J Magarian Blander, Iliyan D Iliev, Danijela Matic Vignjevic, Ana-Maria Lennon-Duménil (2020 Sep 24)
Macrophages Maintain Epithelium Integrity by Limiting Fungal Product Absorption.
Cell : 411-428.e16 : DOI : S0092-8674(20)31090-4

Résumé

The colon is primarily responsible for absorbing fluids. It contains a large number of microorganisms including fungi, which are enriched in its distal segment. The colonic mucosa must therefore tightly regulate fluid influx to control absorption of fungal metabolites, which can be toxic to epithelial cells and lead to barrier dysfunction. How this is achieved remains unknown. Here, we describe a mechanism by which the innate immune system allows rapid quality check of absorbed fluids to avoid intoxication of colonocytes. This mechanism relies on a population of distal colon macrophages that are equipped with « balloon-like » protrusions (BLPs) inserted in the epithelium, which sample absorbed fluids. In the absence of macrophages or BLPs, epithelial cells keep absorbing fluids containing fungal products, leading to their death and subsequent loss of epithelial barrier integrity. These results reveal an unexpected and essential role of macrophages in the maintenance of colon-microbiota interactions in homeostasis. VIDEO ABSTRACT.

Anita Kumari, Judith Pineau, Ana-Maria Lennon-Duménil, Martial Balland, Paolo Pierobon (2020 Aug 11)
Traction Force Microscopy to Study B Lymphocyte Activation.
Journal of visualized experiments : JoVE : DOI : 10.3791/60947

Résumé

Traction force microscopy (TFM) enables the measurement of forces produced by a cell on a substrate. This technique infers traction force measurements from an experimentally observed displacement field produced by a cell pulling on an elastic substrate. Here, we adapted TFM to investigate the spatial and temporal structure of the force field exerted by B cells when activated by antigen engagement of the B cell receptor. Gel rigidity, bead density, and protein functionalization must be optimized for the study of relatively small cells (~ 6 µm) that interact with, and respond specifically to ligands for cell surface receptors.

Yessia Hidalgo, Sarah Núñez, Maria Jose Fuenzalida, Felipe Flores-Santibáñez, Pablo J Sáez, Jessica Dorner, Ana-Maria Lennon-Dumenil, Victor Martínez, Emmanuel Zorn, Mario Rosemblatt, Daniela Sauma, Maria Rosa Bono (2020 May 16)
Thymic B Cells Promote Germinal Center-Like Structures and the Expansion of

Follicular Helper T Cells in Lupus-Prone Mice.
Frontiers in immunology : 696 : DOI : 10.3389/fimmu.2020.00696

Résumé

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the activation of autoreactive T and B cells, autoantibody production, and immune complex deposition in various organs. Previous evidence showed abnormal accumulation of B cells in the thymus of lupus-prone mice, but the role of this population in the progression of the disease remains mostly undefined. Here we analyzed the spatial distribution, function, and properties of this thymic B cell population in the BWF1 murine model of SLE. We found that in diseased animals, thymic B cells proliferate, and cluster in structures that resemble ectopic germinal centers. Moreover, we detected antibody-secreting cells in the thymus of diseased-BWF1 mice that produce anti-dsDNA IgG autoantibodies. We also found that thymic B cells from diseased-BWF1 mice induced the differentiation of thymocytes to follicular helper T cells (T). These data suggest that the accumulation of B cells in the thymus of BWF1 mice results in the formation of germinal center-like structures and the expansion of a T population, which may, in turn, activate and differentiate B cells into autoreactive plasma cells. Therefore, the thymus emerges as an important niche that supports the maintenance of the pathogenic humoral response in the development of murine SLE.

Hélène D Moreau, Ana-Maria Lennon-Duménil, Paolo Pierobon (2020 Mar 4)
« If you please… draw me a cell ». Insights from immune cells.
Journal of cell science : DOI : jcs244806

Résumé

Studies in recent years have shed light on the particular features of cytoskeleton dynamics in immune cells, challenging the classical picture drawn from typical adherent cell lines. New mechanisms linking the dynamics of the membrane-cytoskeleton interface to the mechanical properties of immune cells have been uncovered and shown to be essential for immune surveillance functions. In this Essay, we discuss these features, and propose immune cells as a new playground for cell biologists who try to understand how cells adapt to different microenvironments to fulfil their functions efficiently.

Sandra Sofía Edwards-Jorquera, Floris Bosveld, Yohanns A Bellaïche, Ana-María Lennon-Duménil, Álvaro Glavic (2020 Jan 16)
Trpml controls actomyosin contractility and couples migration to phagocytosis in fly macrophages.
The Journal of cell biology : DOI : e201905228

Résumé

Phagocytes use their actomyosin cytoskeleton to migrate as well as to probe their

environment by phagocytosis or macropinocytosis. Although migration and extracellular material uptake have been shown to be coupled in some immune cells, the mechanisms involved in such coupling are largely unknown. By combining time-lapse imaging with genetics, we here identify the lysosomal Ca2+ channel Trpml as an essential player in the coupling of cell locomotion and phagocytosis in hemocytes, the Drosophila macrophage-like immune cells. Trpml is needed for both hemocyte migration and phagocytic processing at distinct subcellular localizations: Trpml regulates hemocyte migration by controlling actomyosin contractility at the cell rear, whereas its role in phagocytic processing lies near the phagocytic cup in a myosin-independent fashion. We further highlight that Vamp7 also regulates phagocytic processing and locomotion but uses pathways distinct from those of Trpml. Our results suggest that multiple mechanisms may have emerged during evolution to couple phagocytic processing to cell migration and facilitate space exploration by immune cells.

Année de publication : 2019

Stankevicins L, Ecker N, Terriac E, Maiuri P, Schoppmeyer R, Vargas P, Lennon-Duménil AM, Piel M, Qu B, Hoth M, Kruse K, Lautenschläger F. (2020 Jan 14)

Deterministic actin waves as generators of cell polarization cues.

Proceedings of the National Academy of Sciences : 117 : Proc Natl Acad Sci U S A. 2020 Jan 14;117(2):826-835. doi: 10.1073/pnas.1907845117. Epub 2019 Dec 27. : 826,835 : DOI : 10.1073/pnas.1907845117

Résumé

Dendritic cells « patrol » the human body to detect pathogens. In their search, dendritic cells perform a random walk by amoeboid migration. The efficiency of pathogen detection depends on the properties of the random walk. It is not known how the dendritic cells control these properties. Here, we quantify dendritic cell migration under well-defined 2-dimensional confinement and in a 3-dimensional collagen matrix through recording their long-term trajectories. We find 2 different migration states: persistent migration, during which the dendritic cells move along curved paths, and diffusive migration, which is characterized by successive sharp turns. These states exhibit differences in the actin distributions. Our theoretical and experimental analyses indicate that this kind of motion can be generated by spontaneous actin polymerization waves that contribute to dendritic cell polarization and migration. The relative distributions of persistent and diffusive migration can be changed by modification of the molecular actin filament nucleation and assembly rates. Thus, dendritic cells can control their migration patterns and adapt to specific environments. Our study offers an additional perspective on how dendritic cells tune their searches for pathogens.

Anita Kumari, Judith Pineau, Pablo J Sáez, Mathieu Maurin, Danielle Lankar, Mabel San Roman, Katharina Hennig, Vanessa F Boura, Raphael Voituriez, Mikael C I Karlsson, Martial Balland, Ana-Maria Lennon Dumenil, Paolo Pierobon (2019 Jun 30)

Actomyosin-driven force patterning controls endocytosis at the immune synapse.
Nature communications : 2870 : DOI : 10.1038/s41467-019-10751-7

Résumé

An important channel of cell-to-cell communication is direct contact. The immune synapse is a paradigmatic example of such type of interaction: it forms upon engagement of antigen receptors in lymphocytes by antigen-presenting cells and allows the local exchange of molecules and information. Although mechanics has been shown to play an important role in this process, how forces organize and impact on synapse function is unknown. We find that mechanical forces are spatio-temporally patterned at the immune synapse: global pulsatile myosin II-driven tangential forces are observed at the synapse periphery while localised forces generated by invadosome-like F-actin protrusions are detected at its centre. Noticeably, we observe that these force-producing actin protrusions constitute the main site of antigen extraction and endocytosis and require myosin II contractility to form. The interplay between global and local forces dictated by the organization of the actomyosin cytoskeleton therefore controls endocytosis at the immune synapse.

Juan José Sáez, Jheimmy Diaz, Jorge Ibañez, Juan Pablo Bozo, Fernanda Cabrera Reyes, Martina Alamo, François-Xavier Gobert, Dorian Obino, María Rosa Bono, Ana-María Lennon-Duménil, Charles Yeaman, María-Isabel Yuseff (2019 Jun 15)

The exocyst controls lysosome secretion and antigen extraction at the immune synapse of B cells.
The Journal of cell biology : 2247-2264 : DOI : 10.1083/jcb.201811131

Résumé

B lymphocytes capture antigens from the surface of presenting cells by forming an immune synapse. Local secretion of lysosomes, which are guided to the synaptic membrane by centrosome repositioning, can facilitate the extraction of immobilized antigens. However, the molecular basis underlying their delivery to precise domains of the plasma membrane remains elusive. Here we show that microtubule stabilization, triggered by engagement of the B cell receptor, acts as a cue to release centrosome-associated Exo70, which is redistributed to the immune synapse. This process is coupled to the recruitment and activation of GEF-H1, which is required for assembly of the exocyst complex, used to promote tethering and fusion of lysosomes at the immune synapse. B cells silenced for GEF-H1 or Exo70 display defective lysosome secretion, which results in impaired antigen extraction and presentation. Thus, centrosome repositioning coupled to changes in microtubule stability orchestrates the spatial-temporal distribution of the exocyst complex to promote polarized lysosome secretion at the immune synapse.

Juan José Sáez, Ana-María Lennon-Duménil, María-Isabel Yuseff (2019 Jun 1)

Studying MHC Class II Presentation of Immobilized Antigen by B Lymphocytes.
Methods in molecular biology (Clifton, N.J.) : 419-437 : DOI : 10.1007/978-1-4939-9450-2_29

Résumé

The ability of B lymphocytes to capture external antigens (Ag) and present them as peptide fragments, loaded on major histocompatibility complex (MHC) class II molecules, to CD4 T cells is a crucial part of the adaptive immune response. This allows for T-B cooperation, a cellular communication that is required for B cells to develop into germinal centers (GC) and form mature high affinity antibody producing cells and to further develop B cell memory. MHC class II antigen presentation by B lymphocytes is a multistep process involving (1) Recognition and capture of external Ag by B lymphocytes through their B cell receptor (BCR), (2) Ag processing, which comprises the degradation of Ag in internal compartments within the B cell and loading of the corresponding peptide fragments on MHC class II molecules, and (3) Presentation of MHCII-peptide complexes to CD4 T cells. Here, we describe how to study the biochemical and morphological changes that occur in B lymphocytes at these three major levels.

Lucie Barbier, Pablo J Sáez, Rafaele Attia, Ana-Maria Lennon-Duménil, Ido Lavi, Matthieu Piel, Pablo Vargas (2019 Apr 30)
Myosin II Activity Is Selectively Needed for Migration in Highly Confined Microenvironments in Mature Dendritic Cells.
Frontiers in immunology : 747 : DOI : 10.3389/fimmu.2019.00747

Résumé

Upon infection, mature dendritic cells (mDCs) migrate from peripheral tissue to lymph nodes (LNs) to activate T lymphocytes and initiate the adaptive immune response. This fast and tightly regulated process is tuned by different microenvironmental factors, such as the physical properties of the tissue. Mechanistically, mDCs migration mostly relies on acto-myosin flow and contractility that depend on non-muscular Myosin IIA (MyoII) activity. However, the specific contribution of this molecular motor for mDCs navigation in complex microenvironments has yet to be fully established. Here, we identified a specific role of MyoII activity in the regulation of mDCs migration in highly confined microenvironments. Using microfluidic systems, we observed that during mDCs chemotaxis in 3D collagen gels under defined CCL21 gradients, MyoII activity was required to sustain their fast speed but not to orientate them toward the chemokine. Indeed, despite the fact that mDCs speed declined, these cells still migrated through the 3D gels, indicating that this molecular motor has a discrete function during their motility in this irregular microenvironment. Consistently, using microchannels of different sizes, we found that MyoII activity was essential to maintain fast cell speed specifically under strong confinement. Analysis of cell motility through micrometric holes further demonstrated that cell contractility facilitated mDCs passage only over very small gaps. Altogether, this work highlights that high contractility acts as an adaptation mechanism exhibited by mDCs to optimize their motility in restricted landscapes. Hence, MyoII activity ultimately facilitates their navigation in highly confined areas of structurally irregular tissues, contributing to the fine-tuning of their homing to LNs to initiate

adaptive immune responses.

Hélène D Moreau, Carles Blanch-Mercader, Rafaele Attia, Mathieu Maurin, Zahraa Alraies, Doriane Sanséau, Odile Malbec, Maria-Graciela Delgado, Philippe Bousso, Jean-François Joanny, Raphaël Voituriez, Matthieu Piel, Ana-Maria Lennon-Duménil (2019 Apr 16)
Macropinocytosis Overcomes Directional Bias in Dendritic Cells Due to Hydraulic Resistance and Facilitates Space Exploration.
Developmental cell : 171-188.e5 : DOI : S1534-5807(19)30235-7

Résumé

The migration of immune cells can be guided by physical cues imposed by the environment, such as geometry, rigidity, or hydraulic resistance (HR). Neutrophils preferentially follow paths of least HR in vitro, a phenomenon known as barotaxis. The mechanisms and physiological relevance of barotaxis remain unclear. We show that barotaxis results from the amplification of a small force imbalance by the actomyosin cytoskeleton, resulting in biased directional choices. In immature dendritic cells (DCs), actomyosin is recruited to the cell front to build macropinosomes. These cells are therefore insensitive to HR, as macropinocytosis allows fluid transport across these cells. This may enhance their space exploration capacity in vivo. Conversely, mature DCs down-regulate macropinocytosis and are thus barotactic. Modeling suggests that HR may help guide these cells to lymph nodes where they initiate immune responses. Hence, DCs can either overcome or capitalize on the physical obstacles they encounter, helping their immune-surveillance function.