Year of publication 2017

Perrine Paul-Gilloteaux, Xavier Heiligenstein, Martin Belle, Marie-Charlotte Domart, Banafshe Larijani, Lucy Collinson, Graça Raposo, Jean Salamero (2017 Feb 1)
eC-CLEM: flexible multidimensional registration software for correlative microscopies.
Nature methods : 102-103 : DOI : 10.1038/nmeth.4170

Summary

Year of publication 2016

Carlos A Niño, David Guet, Alexandre Gay, Sergine Brutus, Frédéric Jourquin, Shweta Mendiratta, Jean Salamero, Vincent Géli, Catherine Dargemont (2016 Jan 20)
Posttranslational marks control architectural and functional plasticity of the nuclear pore complex basket.
The Journal of cell biology : 167-80 : DOI : 10.1083/jcb.201506130

Summary

The nuclear pore complex (NPC) serves as both the unique gate between the nucleus and the cytoplasm and a major platform that coordinates nucleocytoplasmic exchanges, gene expression, and genome integrity. To understand how the NPC integrates these functional constraints, we dissected here the posttranslational modifications of the nuclear basket protein Nup60 and analyzed how they intervene to control the plasticity of the NPC. Combined approaches highlight the role of monoubiquitylation in regulating the association dynamics of Nup60 and its partner, Nup2, with the NPC through an interaction with Nup84, a component of the Y complex. Although major nuclear transport routes are not regulated by Nup60 modifications, monoubiquitylation of Nup60 is stimulated upon genotoxic stress and regulates the DNA-damage response and telomere repair. Together, these data reveal an original mechanism contributing to the plasticity of the NPC at a molecular-organization and functional level.

Year of publication 2015

David Guet, Laura T Burns, Suman Maji, Jérôme Boulanger, Pascal Hersen, Susan R Wente, Jean Salamero, Catherine Dargemont (2015 Nov 20)
Combining Spinach-tagged RNA and gene localization to image gene expression in live yeast.
Nature communications : 8882 : DOI : 10.1038/ncomms9882

Summary

Although many factors required for the formation of export-competent mRNPs have been described, an integrative view of the spatiotemporal coordinated cascade leading mRNPs from their site of transcription to their site of nuclear exit, at a single cell level, is still partially missing due to technological limitations. Here we report that the RNA Spinach aptamer is a powerful tool for mRNA imaging in live S. cerevisiae with high spatial-temporal resolution and no perturbation of the mRNA biogenesis properties. Dedicated image processing workflows are developed to allow detection of very low abundance of transcripts, accurate quantitative dynamic studies, as well as to provide a localization precision close to 100 nm at consistent time scales. Combining these approaches has provided a state-of-the-art analysis of the osmotic shock response in live yeast by localizing induced transcription factors, target gene loci and corresponding transcripts.

Tianyi Yuan, Lin Liu, Yongdeng Zhang, Lisi Wei, Shiqun Zhao, Xiaolu Zheng, Xiaoshuai Huang, Jerome Boulanger, Charles Gueudry, Jingze Lu, Lihan Xie, Wen Du, Weijian Zong, Lu Yang, Jean Salamero, Yanmei Liu, Liangyi Chen (2015 Oct 7)

Diacylglycerol Guides the Hopping of Clathrin-Coated Pits along Microtubules for Exo-Endocytosis Coupling.

Developmental cell : 120-30 : DOI : 10.1016/j.devcel.2015.09.004

Summary

Many receptor-mediated endocytic processes are mediated by constitutive budding of clathrin-coated pits (CCPs) at spatially randomized sites before slowly pinching off from the plasma membrane (60-100 s). In contrast, clathrin-mediated endocytosis (CME) coupled with regulated exocytosis in excitable cells occurs at peri-exocytic sites shortly after vesicle fusion (~10 s). The molecular mechanism underlying this spatiotemporal coupling remains elusive. We show that coupled endocytosis makes use of pre-formed CCPs, which hop to nascent fusion sites nearby following vesicle exocytosis. A dynamic cortical microtubular network, anchored at the cell surface by the cytoplasmic linker-associated protein on microtubules and the LL5β/ELKS complex on the plasma membrane, provides the track for CCP hopping. Local diacylglycerol gradients generated upon exocytosis guide the direction of hopping. Overall, the CCP-cytoskeleton-lipid interaction demonstrated here mediates exocytosis-coupled fast recycling of both plasma membrane and vesicular proteins, and it is required for the sustained exocytosis during repetitive stimulations.

Antoine Basset, Jérôme Boulanger, Jean Salamero, Patrick Bouthemy, Charles Kervrann (2015 Sep 10)

Adaptive Spot Detection With Optimal Scale Selection in Fluorescence Microscopy Images.

IEEE transactions on image processing : a publication of the IEEE Signal Processing Society : 4512-27 : DOI : 10.1109/TIP.2015.2450996

Summary

Accurately detecting subcellular particles in fluorescence microscopy is of primary interest

for further quantitative analysis such as counting, tracking, or classification. Our primary goal is to segment vesicles likely to share nearly the same size in fluorescence microscopy images. Our method termed adaptive thresholding of Laplacian of Gaussian (LoG) images with autoselected scale (ATLAS) automatically selects the optimal scale corresponding to the most frequent spot size in the image. Four criteria are proposed and compared to determine the optimal scale in a scale-space framework. Then, the segmentation stage amounts to thresholding the LoG of the intensity image. In contrast to other methods, the threshold is locally adapted given a probability of false alarm (PFA) specified by the user for the whole set of images to be processed. The local threshold is automatically derived from the PFA value and local image statistics estimated in a window whose size is not a critical parameter. We also propose a new data set for benchmarking, consisting of six collections of one hundred images each, which exploits backgrounds extracted from real microscopy images. We have carried out an extensive comparative evaluation on several data sets with ground-truth, which demonstrates that ATLAS outperforms existing methods. ATLAS does not need any fine parameter tuning and requires very low computation time. Convincing results are also reported on real total internal reflection fluorescence microscopy images.

Romain Pruvost, Jérôme Boulanger, Bastien Léger, Anne Ponchel, Eric Monflier, Mathias Ibert, André Mortreux, Mathieu Sauthier (2015 Jun 5)

Biphasic Palladium-Catalyzed Hydroesterification in a Polyol Phase: Selective Synthesis of Derived Monoesters.

ChemSusChem : 2133-7 : DOI : 10.1002/cssc.201403397

Summary

The palladium-catalyzed hydroesterification reaction was performed with polyols and olefins in a liquid/liquid biphasic system composed of unreacted polyol on the one hand and apolar reaction products/organic solvents on the other hand. The palladium-based catalyst was immobilized in the polyol phase thanks to the use of cationic triarylphosphines possessing pendent protonated amino groups in the acidic reaction medium or to the sulfonated phosphine TPPTS (trisodium triphenylphosphine-3,3′,3″-trisulfonate). Owing to the insolubility of the products in the catalytic phase, this approach allowed the synthesis of monoesters of polyols with high selectivities as well as the easy separation of the catalyst through simple decantation.

V Fraisier, G Clouvel, A Jasaitis, A Dimitrov, T Piolot, J Salamero (2015 May 6)

Adaptive optics in spinning disk microscopy: improved contrast and brightness by a simple and fast method.

Journal of microscopy : 219-27 : DOI : 10.1111/jmi.12256

Summary

Multiconfocal microscopy gives a good compromise between fast imaging and reasonable resolution. However, the low intensity of live fluorescent emitters is a major limitation to this

technique. Aberrations induced by the optical setup, especially the mismatch of the refractive index and the biological sample itself, distort the point spread function and further reduce the amount of detected photons. Altogether, this leads to impaired image quality, preventing accurate analysis of molecular processes in biological samples and imaging deep in the sample. The amount of detected fluorescence can be improved with adaptive optics. Here, we used a compact adaptive optics module (adaptive optics box for sectioning optical microscopy), which was specifically designed for spinning disk confocal microscopy. The module overcomes undesired anomalies by correcting for most of the aberrations in confocal imaging. Existing aberration detection methods require prior illumination, which bleaches the sample. To avoid multiple exposures of the sample, we established an experimental model describing the depth dependence of major aberrations. This model allows us to correct for those aberrations when performing a z-stack, gradually increasing the amplitude of the correction with depth. It does not require illumination of the sample for aberration detection, thus minimizing photobleaching and phototoxicity. With this model, we improved both signal-to-background ratio and image contrast. Here, we present comparative studies on a variety of biological samples.

Year of publication 2014

Thierry Pécot, Patrick Bouthemy, Jérôme Boulanger, Anatole Chessel, Sabine Bardin, Jean Salamero, Charles Kervrann (2014 Dec 12)

Background fluorescence estimation and vesicle segmentation in live cell imaging with conditional random fields.

IEEE transactions on image processing : a publication of the IEEE Signal Processing Society : 667-80 : DOI : 10.1109/TIP.2014.2380178

Summary

Image analysis applied to fluorescence live cell microscopy has become a key tool in molecular biology since it enables to characterize biological processes in space and time at the subcellular level. In fluorescence microscopy imaging, the moving tagged structures of interest, such as vesicles, appear as bright spots over a static or nonstatic background. In this paper, we consider the problem of vesicle segmentation and time-varying background estimation at the cellular scale. The main idea is to formulate the joint segmentation-estimation problem in the general conditional random field framework. Furthermore, segmentation of vesicles and background estimation are alternatively performed by energy minimization using a min cut-max flow algorithm. The proposed approach relies on a detection measure computed from intensity contrasts between neighboring blocks in fluorescence microscopy images. This approach permits analysis of either 2D + time or 3D + time data. We demonstrate the performance of the so-called C-CRAFT through an experimental comparison with the state-of-the-art methods in fluorescence video-microscopy. We also use this method to characterize the spatial and temporal distribution of Rab6 transport carriers at the cell periphery for two different specific adhesion geometries.

Jérôme Boulanger, Charles Gueudry, Daniel Münch, Bertrand Cinquin, Perrine Paul-Gilloteaux, Sabine Bardin, Christophe Guérin, Fabrice Senger, Laurent Blanchoin, Jean Salamero (2014 Nov 17)

Fast high-resolution 3D total internal reflection fluorescence microscopy by incidence angle scanning and azimuthal averaging.

Proceedings of the National Academy of Sciences of the United States of America : 17164-9 : DOI : 10.1073/pnas.1414106111

Summary

Total internal reflection fluorescence microscopy (TIRFM) is the method of choice to visualize a variety of cellular processes in particular events localized near the plasma membrane of live adherent cells. This imaging technique not relying on particular fluorescent probes provides a high sectioning capability. It is, however, restricted to a single plane. We present here a method based on a versatile design enabling fast multiwavelength azimuthal averaging and incidence angles scanning to computationally reconstruct 3D images sequences. We achieve unprecedented 50-nm axial resolution over a range of 800 nm above the coverslip. We apply this imaging modality to obtain structural and dynamical information about 3D actin architectures. We also temporally decipher distinct Rab11a-dependent exocytosis events in 3D at a rate of seven stacks per second.

Xavier Heiligenstein, Ilse Hurbain, Cédric Delevoye, Jean Salamero, Claude Antony, Graca Raposo (2014 Oct 8)

Step by step manipulation of the CryoCapsule with HPM high pressure freezers.

Methods in cell biology : 259-74 : DOI : 10.1016/B978-0-12-801075-4.00012-4

Summary

The CryoCapsule is a tool dedicated to correlative light to electron microscopy experiments. Focused on simplifying the specimen manipulation throughout the entire workflow from live-cell imaging to freeze substitution following cryofixation by high pressure freezing, we introduce here a step by step procedure to use the CryoCapsule either with the high pressure freezing machines: HPM010 or the HPM100.

Romain Pruvost, Jérôme Boulanger, Bastien Léger, Anne Ponchel, Eric Monflier, Mathias Ibert, André Mortreux, Thomas Chenal, Mathieu Sauthier (2014 Sep 12)

Synthesis of 1,4:3,6-dianhydrohexitols diesters from the palladium-catalyzed hydroesterification reaction.

ChemSusChem : 3157-63 : DOI : 10.1002/cssc.201402584

Summary

The hydroesterification of alpha olefins has been used to synthesize diesters from bio-based

secondary diols: isosorbide, isomannide, and isoidide. The reaction was promoted by 0.2% palladium catalyst generated in situ from palladium acetate/triphenylphosphine/para-toluene sulfonic acid. Optimized reaction conditions allowed the selective synthesis of the diesters with high yields and the reaction conditions could be scaled up to the synthesis of hundred grams of diesters from isosorbide and 1-octene with solvent-free conditions.

Carine Rossé, Catalina Lodillinsky, Laetitia Fuhrmann, Maya Nourieh, Pedro Monteiro, Marie Irondelle, Emilie Lagoutte, Sophie Vacher, François Waharte, Perrine Paul-Gilloteaux, Maryse Romao, Lucie Sengmanivong, Mark Linch, Johan van Lint, Graça Raposo, Anne Vincent-Salomon, Ivan Bièche, Peter J Parker, Philippe Chavrier (2014 Apr 21)
Control of MT1-MMP transport by atypical PKC during breast-cancer progression.
Proceedings of the National Academy of Sciences of the United States of America : E1872-9 : DOI : 10.1073/pnas.1400749111

Summary

Dissemination of carcinoma cells requires the pericellular degradation of the extracellular matrix, which is mediated by membrane type 1-matrix metalloproteinase (MT1-MMP). In this article, we report a co-up-regulation and colocalization of MT1-MMP and atypical protein kinase C iota (aPKCι) in hormone receptor-negative breast tumors in association with a higher risk of metastasis. Silencing of aPKC in invasive breast-tumor cell lines impaired the delivery of MT1-MMP from late endocytic storage compartments to the surface and inhibited matrix degradation and invasion. We provide evidence that aPKCι, in association with MT1-MMP-containing endosomes, phosphorylates cortactin, which is present in F-actin-rich puncta on MT1-MMP-positive endosomes and regulates cortactin association with the membrane scission protein dynamin-2. Thus, cell line-based observations and clinical data reveal the concerted activity of aPKC, cortactin, and dynamin-2, which control the trafficking of MT1-MMP from late endosome to the plasma membrane and play an important role in the invasive potential of breast-cancer cells.

Nicolas Chenouard, Ihor Smal, Fabrice de Chaumont, Martin Maška, Ivo F Sbalzarini, Yuanhao Gong, Janick Cardinale, Craig Carthel, Stefano Coraluppi, Mark Winter, Andrew R Cohen, William J Godinez, Karl Rohr, Yannis Kalaidzidis, Liang Liang, James Duncan, Hongying Shen, Yingke Xu, Klas E G Magnusson, Joakim Jaldén, Helen M Blau, Perrine Paul-Gilloteaux, Philippe Roudot, Charles Kervrann, François Waharte, Jean-Yves Tinevez, Spencer L Shorte, Joost Willemse, Katherine Celler, Gilles P van Wezel, Han-Wei Dan, Yuh-Show Tsai, Carlos Ortiz de Solórzano, Jean-Christophe Olivo-Marin, Erik Meijering (2014 Jan 21)
Objective comparison of particle tracking methods.
Nature methods : 281-9 : DOI : 10.1038/nmeth.2808

Summary

Particle tracking is of key importance for quantitative analysis of intracellular dynamic processes from time-lapse microscopy image data. Because manually detecting and following large numbers of individual particles is not feasible, automated computational methods have been developed for these tasks by many groups. Aiming to perform an objective comparison of methods, we gathered the community and organized an open competition in which participating teams applied their own methods independently to a commonly defined data set including diverse scenarios. Performance was assessed using commonly defined measures. Although no single method performed best across all scenarios, the results revealed clear differences between the various approaches, leading to notable practical conclusions for users and developers.

Year of publication 2013

Pedro Monteiro, Carine Rossé, Antonio Castro-Castro, Marie Irondelle, Emilie Lagoutte, Perrine Paul-Gilloteaux, Claire Desnos, Etienne Formstecher, François Darchen, David Perrais, Alexis Gautreau, Maud Hertzog, Philippe Chavrier (2013 Dec 18)
Endosomal WASH and exocyst complexes control exocytosis of MT1-MMP at invadopodia.
The Journal of cell biology : 1063-79

Summary

Remodeling of the extracellular matrix by carcinoma cells during metastatic dissemination requires formation of actin-based protrusions of the plasma membrane called invadopodia, where the trans-membrane type 1 matrix metalloproteinase (MT1-MMP) accumulates. Here, we describe an interaction between the exocyst complex and the endosomal Arp2/3 activator Wiskott-Aldrich syndrome protein and Scar homolog (WASH) on MT1-MMP–containing late endosomes in invasive breast carcinoma cells. We found that WASH and exocyst are required for matrix degradation by an exocytic mechanism that involves tubular connections between MT1-MMP–positive late endosomes and the plasma membrane in contact with the matrix. This ensures focal delivery of MT1-MMP and supports pericellular matrix degradation and tumor cell invasion into different pathologically relevant matrix environments. Our data suggest a general mechanism used by tumor cells to breach the basement membrane and for invasive migration through fibrous collagen-enriched tissues surrounding the tumor.

Giulia Bertolin, Rosa Ferrando-Miguel, Maxime Jacoupy, Sabine Traver, Karl Grenier, Andrew W Greene, Aurélien Dauphin, François Waharte, Aurélien Bayot, Jean Salamero, Anne Lombès, Anne-Laure Bulteau, Edward A Fon, Alexis Brice, Olga Corti (2013 Oct 24)
The TOMM machinery is a molecular switch in PINK1 and PARK2/PARKIN-

dependent mitochondrial clearance.
Autophagy : 1801-17 : DOI : 10.4161/auto.25884

Summary

Loss-of-function mutations in PARK2/PARKIN and PINK1 cause early-onset autosomal recessive Parkinson disease (PD). The cytosolic E3 ubiquitin-protein ligase PARK2 cooperates with the mitochondrial kinase PINK1 to maintain mitochondrial quality. A loss of mitochondrial transmembrane potential (ΔΨ) leads to the PINK1-dependent recruitment of PARK2 to the outer mitochondrial membrane (OMM), followed by the ubiquitination and proteasome-dependent degradation of OMM proteins, and by the autophagy-dependent clearance of mitochondrial remnants. We showed here that blockade of mitochondrial protein import triggers the recruitment of PARK2, by PINK1, to the TOMM machinery. PD-causing PARK2 mutations weakened or disrupted the molecular interaction between PARK2 and specific TOMM subunits: the surface receptor, TOMM70A, and the channel protein, TOMM40. The downregulation of TOMM40 or its associated core subunit, TOMM22, was sufficient to trigger OMM protein clearance in the absence of PINK1 or PARK2. However, PARK2 was required to promote the degradation of whole organelles by autophagy. Furthermore, the overproduction of TOMM22 or TOMM40 reversed mitochondrial clearance promoted by PINK1 and PARK2 after ΔΨ loss. These results indicated that the TOMM machinery is a key molecular switch in the mitochondrial clearance program controlled by the PINK1-PARK2 pathway. Loss of functional coupling between mitochondrial protein import and the neuroprotective degradation of dysfunctional mitochondria may therefore be a primary pathogenic mechanism in autosomal recessive PD.