
**Physiological alpha5beta1 integrin transmembrane protein extraction, purification and reconstitution into proteo-lipidic nanodiscs bilayer**

*Methods in Molecular Biology on Heterologous Expression of Membrane Proteins*

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Marc Lavigne, Olivier Helynck, Pascal Rigolet, Rofia Boudria-Souilah, Mireille Nowakowski, Bruno Baron, Sébastien Brülé, Sylviane Hoos, Bertrand Raynal, Lionel Guittat, Claire Beauvain, Stéphane Petres, Anton Granzhan, Jean Guillon, Geneviève Pratviel, Marie-Paule Teulade-Fichou, Patrick England, Jean-Louis Mergny, Hélène Munier-Lehmann (2021 Jul 7)

**SARS-CoV-2 Nsp3 unique domain SUD interacts with guanine quadruplexes and G4-ligands inhibit this interaction.**


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V. Kapoor, C. Carabaña (2021 Jul 6)

**Cell Tracking in 3D using deep learning segmentations**

*scipy*

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Although many computational tools have been developed during the past years to automatically detect and track cells, they are optimized to detect cell nuclei with similar shapes and/or cells not clustering together. These existing tools are challenged when tracking fluorescently labelled membranes of cells due to cell’s irregular shape, variability in size and dynamic movement across Z planes making it difficult to detect and track them.

Here we introduce a detailed analysis pipeline to perform segmentation with accurate shape information, combined with BTrackmate, a customized codebase of popular ImageJ/Fiji software Trackmate, to perform cell tracking inside the tissue of interest. We developed VollSeg, a new segmentation method able to detect membrane-labelled cells with low signal-to-noise ratio and dense packing. Finally, we also created an interface in Napari, an Euler angle based viewer, to visualize the tracks along a chosen view making it possible to follow a cell along the plane of motion. Importantly, we provide a detailed protocol to implement this pipeline in a new dataset, together with the required Jupyter notebooks.

M. Plays, S. Müller, R. Rodriguez (2021 Jul 1)
Chemistry and Biology of Ferritin
Metallomics : DOI : 10.1093/mtomcs/mfab021

Summary

Correlative AFM and fluorescence imaging demonstrate nanoscale membrane remodeling and ring-like and tubular structure formation by septins
Nanoscale : DOI : 10.1039/D1NR01978C

Summary

Septins are ubiquitous cytoskeletal filaments that interact with the inner plasma membrane and are essential for cell division in eukaryotes. In cellular contexts, septins are often localized at micrometric gaussian curvatures, where they assemble onto ring-like structures. The behavior of budding yeast septins depends on their specific interaction with inositol phospholipids, enriched at the inner leaflet of the plasma membrane. Septin filaments are built from the non-polar self-assembly of short rods into filaments. However, the molecular mechanisms regulating the interplay with the inner plasma membrane and the resulting interaction with specific curvatures are not fully understood. In this report, we have imaged dynamical molecular assemblies of budding yeast septins on PIP2-
containing supported lipid bilayers using a combination of high-speed AFM and correlative AFM-fluorescence microscopy. Our results clearly demonstrate that septins are able to bind to flat supported lipid bilayers and thereafter induce the remodeling of membranes. Short septin rods (octamers subunits) can indeed destabilize supported lipid bilayers and reshape the membrane to form 3D structures such as rings and tubes, demonstrating that long filaments are not necessary for septin-induced membrane buckling.

Weitao Wang, Kyle N Klein, Karel Proesmans, Hongbo Yang, Claire Marchal, Xiaopeng Zhu, Tyler Borrman, Alex Hastie, Zhiping Weng, John Bechhoefer, Chun-Long Chen, David M Gilbert, Nicholas Rhind (2021 Jun 22)

Genome-wide mapping of human DNA replication by optical replication mapping supports a stochastic model of eukaryotic replication.

Molecular cell: DOI : S1097-2765(21)00408-1

Summary

The heterogeneous nature of eukaryotic replication kinetics and the low efficiency of individual initiation sites make mapping the location and timing of replication initiation in human cells difficult. To address this challenge, we have developed optical replication mapping (ORM), a high-throughput single-molecule approach, and used it to map early-initiation events in human cells. The single-molecule nature of our data and a total of >2,500-fold coverage of the human genome on 27 million fibers averaging ~300 kb in length allow us to identify initiation sites and their firing probability with high confidence. We find that the distribution of human replication initiation is consistent with inefficient, stochastic activation of heterogeneously distributed potential initiation complexes enriched in accessible chromatin. These observations are consistent with stochastic models of initiation-timing regulation and suggest that stochastic regulation of replication kinetics is a fundamental feature of eukaryotic replication, conserved from yeast to humans.

Katrina Cristall, Francois-Clement Bidard, Jean-Yves Pierga, Michael J Rauh, Tatiana Popova, Clara Sebbag, Olivier Lantz, Marc-Henri Stern, Christopher R Mueller (2021 Jun 17)

A DNA methylation-based liquid biopsy for triple-negative breast cancer.

NPJ precision oncology : DOI : 10.1038/s41698-021-00198-9

Summary

Here, we present a next-generation sequencing (NGS) methylation-based blood test called methylation DETEction of Circulating Tumour DNA (mDETECT) designed for the optimal detection and monitoring of metastatic triple-negative breast cancer (TNBC). Based on a
highly multiplexed targeted sequencing approach, this assay incorporates features that offer superior performance and included 53 amplicons from 47 regions. Analysis of a previously characterised cohort of women with metastatic TNBC with limited quantities of plasma (<2 ml) produced an AUC of 0.92 for detection of a tumour with a sensitivity of 76% for a specificity of 100%. mDETECT was quantitative and showed superior performance to an NGS TP53 mutation-based test carried out on the same patients and to the conventional CA15-3 biomarker. mDETECT also functioned well in serum samples from metastatic TNBC patients where it produced an AUC of 0.97 for detection of a tumour with a sensitivity of 93% for a specificity of 100%. An assay for BRCA1 promoter methylation was also incorporated into the mDETECT assay and functioned well but its clinical significance is currently unclear. Clonal Hematopoiesis of Indeterminate Potential was investigated as a source of background in control subjects but was not seen to be significant, though a link to adiposity may be relevant. The mDETECT assay is a liquid biopsy able to quantitatively detect all TNBC cancers and has the potential to improve the management of patients with this disease.

Graça Raposo, Guillaume van Niel, Philip D Stahl (2021 Jun 14)
Extracellular vesicles and homeostasis-An emerging field in bioscience research.

Summary

To keep abreast of developments in the biological sciences and in parallel fields such as medical education, () has created a special collections category, special collections ( SC), that target, among other topics, emerging disciplines in the biomedical sciences. This SC is focused on the emerging field of extracellular vesicles (EVs) and homeostasis. Leading investigators in the biology of EVs around the globe have contributed to this collection of articles that cover the gamut of research activities from biogenesis and secretion to physiological function.

Frequency and Prognostic Impact of Amplifications and Mutations in the European Neuroblastoma Study Group (SIOPEN) High-Risk Neuroblastoma Trial (HR-NBL1).
Journal of clinical oncology : official journal of the American Society of Clinical Oncology :
Summary

In neuroblastoma (NB), the ALK receptor tyrosine kinase can be constitutively activated through activating point mutations or genomic amplification. We studied genetic alterations in high-risk (HR) patients on the HR-NBL1/SIOPEN trial to determine their frequency, correlation with clinical parameters, and prognostic impact.

Daniel Lévy, Aurélie Di Cicco, Aurélie Bertin, Manuela Dezi (2021 Jun 7)

[Cryo-electron microscopy for a new vision of the cell and its components]
Medecine/Sciences : 379-385 : DOI : 10.1051/medsci/2021034

Summary

Cryo-electron microscopy (cryo-EM) is a technique for imaging biological samples that plays a central role in structural biology, with high impact on research fields such as cell and developmental biology, bioinformatics, cell physics and applied mathematics. It allows the determination of structures of purified proteins within cells. This review describes the main recent advances in cryo-EM, illustrated by examples of proteins of biomedical interest, and the avenues for future development.


Nanoscale architecture of a VAP-A-OSBP tethering complex at membrane contact sites
Nature Communications : DOI : 10.1038/s41467-021-23799-1

Summary

Membrane contact sites (MCS) are subcellular regions where two organelles appose their membranes to exchange small molecules, including lipids. structural information on how proteins form MCS is scarce. We designed an in vitro MCS with two membranes and a pair of tethering proteins suitable for cryo-tomography analysis. It includes VAP-A, an ER transmembrane protein interacting with a myriad of cytosolic proteins, and oxysterol-binding protein (OSBP), a lipid transfer protein that transports cholesterol from the ER to the trans Golgi network. We show that VAP-A is a highly flexible protein, allowing formation of MCS of variable intermembrane distance. The tethering part of OSBP contains a central, dimeric, and helical T-shape region. We propose that the molecular flexibility of VAP-A enables the recruitment of partners of different sizes within MCS of adjustable thickness, whereas the T geometry of the OSBP dimer facilitates the movement of the two lipid-transfer domains between membranes.

**Molecular basis of the dual role of the Mlh1-Mlh3 endonuclease in MMR and in meiotic crossover formation.**
*Proceedings of the National Academy of Sciences of the United States of America:* DOI: e2022704118

**Summary**

Méiose et réparation de l’ADN : les chercheurs décryptent l’activité d’un complexe moléculaire spécifique

In budding yeast, the MutL homolog heterodimer Mlh1-Mlh3 (MutLγ) plays a central role in the formation of meiotic crossovers. It is also involved in the repair of a subset of mismatches besides the main mismatch repair (MMR) endonuclease Mlh1-Pms1 (MutLα). The heterodimer interface and endonuclease sites of MutLγ and MutLα are located in their C-terminal domain (CTD). The molecular basis of MutLγ’s dual roles in MMR and meiosis is not known. To better understand the specificity of MutLγ, we characterized the crystal structure of MutLγ(CTD). Although MutLγ(CTD) presents overall similarities with MutLα(CTD), it harbors some rearrangement of the surface surrounding the active site, which indicates altered substrate preference. The last amino acids of Mlh1 participate in the Mlh3 endonuclease site as previously reported for Pms1. We characterized alleles and showed a critical role of this Mlh1 extreme C terminus both in MMR and in meiotic recombination. We showed that the MutLγ(CTD) preferentially binds Holliday junctions, contrary to MutLα(CTD). We characterized Mlh3 positions on the N-terminal domain (NTD) and CTD that could contribute to the positioning of the NTD close to the CTD in the context of the full-length MutLγ. Finally, crystal packing revealed an assembly of MutLγ(CTD) molecules in filament structures. Mutation at the corresponding interfaces reduced crossover formation, suggesting that these superstructures may contribute to the oligomer formation proposed for MutLγ. This study defines clear divergent features between the MutL homologs and identifies, at the molecular level, their specialization toward MMR or meiotic recombination functions.


**Loss of SDHB promotes dysregulated iron homeostasis, oxidative stress and sensitivity to ascorbate**
*Cancer Research:* DOI: 10.1158/0008-5472.CAN-20-2936

**Summary**
Peter Peneder, Adrian M Stütz, Didier Surdez, Manuela Krumbholz, Sabine Semper, Mathieu Chicard, Nathan C Sheffield, Gaelle Pierron, Eve Lapouble, Marcus Tötzl, Bekir Ergüner, Daniele Barreca, André F Rendeiro, Abbas Agaimy, Heidrun Boztug, Gernot Engstler, Michael Dworzak, Marie Bernkopf, Sabine Taschner-Mandl, Inge M Ambros, Ola Myklebost, Perrine Marec-Bérard, Susan Ann Burchill, Bernadette Brennan, Sandra J Strauss, Jeremy Whelan, Gudrun Schleiermacher, Christiane Schaefer, Uta Dirksen, Caroline Hutter, Kjetil Boye, Peter F Ambros, Olivier Delattre, Markus Metzler, Christoph Bock, Eleni M Tomazou (2021 May 29)
Multimodal analysis of cell-free DNA whole-genome sequencing for pediatric cancers with low mutational burden.
*Nature communications* : 3230 : DOI : 10.1038/s41467-021-23445-w

**Summary**

Sequencing of cell-free DNA in the blood of cancer patients (liquid biopsy) provides attractive opportunities for early diagnosis, assessment of treatment response, and minimally invasive disease monitoring. To unlock liquid biopsy analysis for pediatric tumors with few genetic aberrations, we introduce an integrated genetic/epigenetic analysis method and demonstrate its utility on 241 deep whole-genome sequencing profiles of 95 patients with Ewing sarcoma and 31 patients with other pediatric sarcomas. Our method achieves sensitive detection and classification of circulating tumor DNA in peripheral blood independent of any genetic alterations. Moreover, we benchmark different metrics for cell-free DNA fragmentation analysis, and we introduce the LIQUORICE algorithm for detecting circulating tumor DNA based on cancer-specific chromatin signatures. Finally, we combine several fragmentation-based metrics into an integrated machine learning classifier for liquid biopsy analysis that exploits widespread epigenetic deregulation and is tailored to cancers with low mutation rates. Clinical associations highlight the potential value of cfDNA fragmentation patterns as prognostic biomarkers in Ewing sarcoma. In summary, our study provides a comprehensive analysis of circulating tumor DNA beyond recurrent genetic aberrations, and it renders the benefits of liquid biopsy more readily accessible for childhood cancers.

Linh Le, Julia Sirés-Campos, Graça Raposo, Cédric Delevoye, Michael S Marks (2021 May 22)
Melanosome biogenesis in the pigmentation of mammalian skin.
*Integrative and comparative biology* : DOI : icab078

**Summary**

Melanins, the main pigments of the skin and hair in mammals, are synthesized within membrane-bound organelles of melanocytes called melanosomes. Melanosome structure and function are determined by a cohort of resident transmembrane proteins, many of which are expressed only in pigment cells, that localize specifically to melanosomes. Defects in the genes that encode melanosome-specific proteins or components of the machinery required for their transport in and out of melanosomes underlie various forms of ocular or oculocutaneous albinism, characterized by hypopigmentation of the hair, skin and eyes and
by visual impairment. We review major components of melanosomes, including the enzymes that catalyze steps in melanin synthesis from tyrosine precursors, solute transporters that allow these enzymes to function, and structural proteins that underlie melanosome shape and melanin deposition. We then review the molecular mechanisms by which these components are biosynthetically delivered to newly forming melanosomes—many of which are shared by other cell types that generate cell type-specific lysosome-related organelles. We also highlight unanswered questions that need to be addressed by future investigation.