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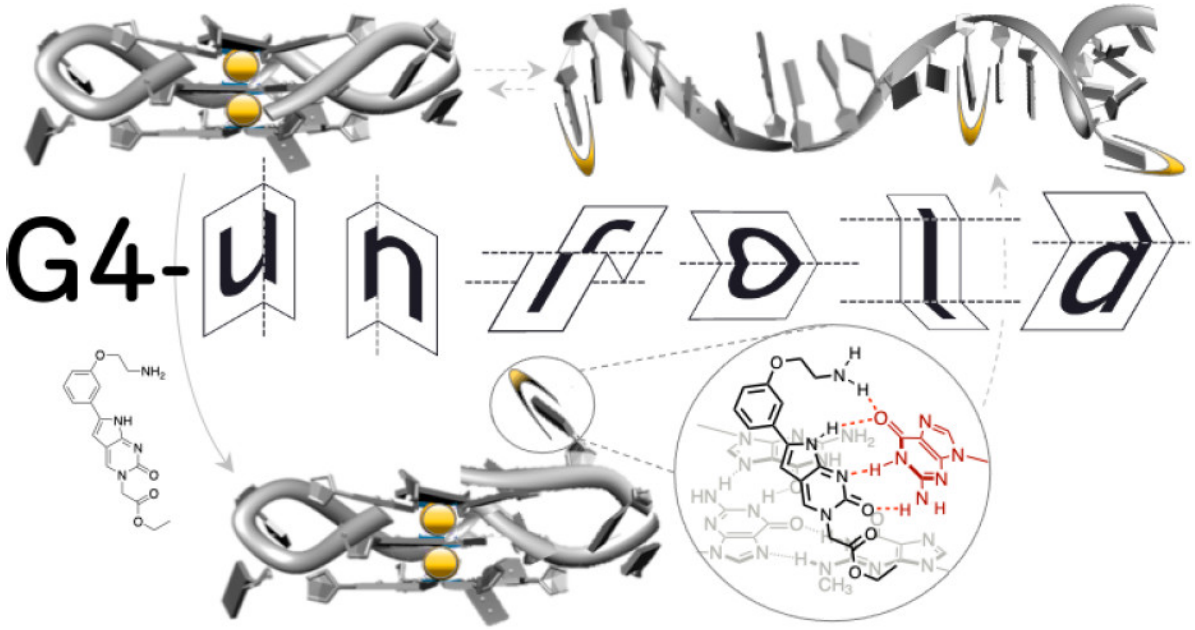
Jérémie Mitteaux, Pauline Lejault, Filip Wojciechowski, Alexandra Joubert, Julien Boudon, Nicolas Desbois, Claude P. Gros, Robert H. E. Hudson, Jean-Baptiste Boulé, Anton Granzhan, David Monchaud (2021 Aug 4)

Identifying G-Quadruplex-DNA-Disrupting Small Molecules

Journal of the American Chemical Society : 143 : 12567–12577 : DOI : 10.1021/jacs.1c04426

Summary

The quest for small molecules that strongly bind to G-quadruplex-DNA (G4), so-called G4 ligands, has invigorated the G4 research field from its very inception. Massive efforts have been invested to discover or rationally design G4 ligands, evaluate their G4-interacting properties in vitro through a series of now widely accepted and routinely implemented assays, and use them as innovative chemical biology tools to interrogate cellular networks that might involve G4s. In sharp contrast, only uncoordinated efforts aimed at developing small molecules that destabilize G4s have been invested to date, even though it is now recognized that such molecular tools would have tremendous application in neurobiology as many genetic and age-related diseases are caused by an overrepresentation of G4s. Herein, we report on our efforts to develop in vitro assays to reliably identify molecules able to destabilize G4s. This workflow comprises the newly designed G4-unfold assay, adapted from the G4-helicase assay implemented with Pif1, as well as a series of biophysical and biochemical techniques classically used to study G4/ligand interactions (CD, UV–vis, PAGE, and FRET-melting), and a qPCR stop assay, adapted from a *Taq*-based protocol recently used to identify G4s in the genomic DNA of *Schizosaccharomyces pombe*. This unique, multipronged approach leads to the characterization of a phenylpyrrolocytosine (PhpC)-based G-clamp analog as a prototype of G4-disrupting small molecule whose properties are validated through many different and complementary in vitro evaluations.



Estelle Dransart, Aurélie Di Cicco, Ahmed El Marjou, Daniel Lévy, Staffan Johansson, Ludger Johannes, Massiullah Shafaq-Zadah (2021 Jul 14)
Physiological alpha5beta1 integrin transmembrane protein extraction, purification and reconstitution into proteo-lipidic nanodiscs bilayer
Methods in Molecular Biology on Heterologous Expression of Membrane ProteinsMethods in Molecular Biology on Heterologous Expression of Membrane Proteins

Summary

Shengjun Tan, Huijing Ma, Jinbo Wang, Man Wang, Mengxia Wang, Haodong Yin, Yaqiong Zhang, Xinying Zhang, Jieyu Shen, Danyang Wang, Graham L Banes, Zhihua Zhang, Jianmin Wu, Xun Huang, Hua Chen, Siqin Ge, Chun-Long Chen, Yong E Zhang (2021 Jul 14)
DNA transposons mediate duplications via transposition-independent and -dependent mechanisms in metazoans.
Nature communications : 4280 : DOI : 10.1038/s41467-021-24585-9

Summary

Despite long being considered as “junk”, transposable elements (TEs) are now accepted as catalysts of evolution. One example is Mutator-like elements (MULEs, one type of terminal inverted repeat DNA TEs, or TIR TEs) capturing sequences as Pack-MULEs in plants. However, their origination mechanism remains perplexing, and whether TIR TEs mediate duplication in animals is almost unexplored. Here we identify 370 Pack-TIRs in 100 animal reference genomes and one Pack-TIR (Ssk-FB4) family in fly populations. We find that single-copy Pack-

TIRs are mostly generated via transposition-independent gap filling, and multicopy Pack-TIRs are likely generated by transposition after replication fork switching. We show that a proportion of Pack-TIRs are transcribed and often form chimeras with hosts. We also find that Ssk-FB4s represent a young protein family, as supported by proteomics and signatures of positive selection. Thus, TIR TEs catalyze new gene structures and new genes in animals via both transposition-independent and -dependent mechanisms.

Iv François, Martins C., Castro-Linares G., Taveneau C., Barbier P., Verdier-Pinard P., Camoin L., Audebert S., Tsai F.C., Ramond L., Llewellyn A., Belhabib M., Nakazawa K., Di Cicco A., Vincentelli R., Wenger J., Cabantous S., Koenderink G*. H., Bertin A*., Mavrakis M*. (2021 Jul 8)
Insights into animal septins using recombinant human septin octamers with distinct SEPT9 isoforms
Journal of Cell Science : DOI : 10.1242/jcs.258484

Summary

Septin GTP-binding proteins contribute essential biological functions that range from the establishment of cell polarity to animal tissue morphogenesis. Human septins in cells form hetero-octameric septin complexes containing the ubiquitously expressed SEPT9. Despite the established role of SEPT9 in mammalian development and human pathophysiology, biochemical and biophysical studies have relied on monomeric SEPT9 thus not recapitulating its native assembly into hetero-octameric complexes. We established a protocol that enabled the first-time isolation of recombinant human septin octamers containing distinct SEPT9 isoforms. A combination of biochemical and biophysical assays confirmed the octameric nature of the isolated complexes in solution. Reconstitution studies showed that octamers with either a long or a short SEPT9 isoform form filament assemblies, and can directly bind and cross-link actin filaments, raising the possibility that septin-decorated actin structures in cells reflect direct actin-septin interactions. Recombinant SEPT9-containing octamers will make it possible to design cell-free assays to dissect the complex interactions of septins with cell membranes and the actin/microtubule cytoskeleton.

Marc Lavigne, Olivier Helynck, Pascal Rigolet, Rofia Boudria-Souilah, Mireille Nowakowski, Bruno Baron, Sébastien Brülé, Sylviane Hoos, Bertrand Raynal, Lionel Guittat, Claire Beauvineau, 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.
Nucleic Acids Research : 49 : 7695–7712 : DOI : 10.1093/nar/gkab571

Summary

The multidomain non-structural protein 3 (Nsp3) is the largest protein encoded by coronavirus (CoV) genomes and several regions of this protein are essential for viral

replication. Of note, SARS-CoV Nsp3 contains a SARS-Unique Domain (SUD), which can bind Guanine-rich non-canonical nucleic acid structures called G-quadruplexes (G4) and is essential for SARS-CoV replication. We show herein that the SARS-CoV-2 Nsp3 protein also contains a SUD domain that interacts with G4s. Indeed, interactions between SUD proteins and both DNA and RNA G4s were evidenced by G4 pull-down, Surface Plasmon Resonance and Homogenous Time Resolved Fluorescence. These interactions can be disrupted by mutations that prevent oligonucleotides from folding into G4 structures and, interestingly, by molecules known as specific ligands of these G4s. Structural models for these interactions are proposed and reveal significant differences with the crystallographic and modeled 3D structures of the SARS-CoV SUD-NM/G4 interaction. Altogether, our results pave the way for further studies on the role of SUD/G4 interactions during SARS-CoV-2 replication and the use of inhibitors of these interactions as potential antiviral compounds.

V. Kapoor, C. Carabaña (2021 Jul 6)
Cell Tracking in 3D using deep learning segmentations
scipy

Summary

Live-cell imaging is a highly used technique to study cell migration and dynamics over time. 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

Vial Anthony, Taveneau Cyntia, Costa Luca , Chauvin Brieuc , Nasrallah Hussein , Godefroy Cédric, Dosset Patrice , Isambert Hervé , Ngo Kien Xuan, Mangenot Stéphanie , Levy Daniel , Bertin Aurélie* , Milhiet Pierre-Emmanuel * (2021 Jun 29)

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 : 53 : 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.
FASEB bioAdvances : 456-458 : DOI : 10.1096/fba.2021-00009

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.

Angela Bellini, Ulrike Pötschger, Virginie Bernard, Eve Lapouble, Sylvain Baulande, Peter F Ambros, Nathalie Auger, Klaus Beiske, Marie Bernkopf, David R Betts, Jaydutt Bhalshankar, Nick Bown, Katleen de Preter, Nathalie Clément, Valérie Combaret, Jaime Font de Mora, Sally L George, Irene Jiménez, Marta Jeison, Barbara Marques, Tommy Martinsson, Katia Mazzocco, Martina Morini, Annick Mühlethaler-Mottet, Rosa Noguera, Gaelle Pierron, Maria Rossing, Sabine Taschner-Mandl, Nadine Van Roy, Ales Vicha, Louis Chesler, Walentyna Balwierz, Victoria Castel, Martin Elliott, Per Kogner, Geneviève Laureys, Roberto Luksch, Josef Malis, Maja Popovic-Beck, Shifra Ash, Olivier Delattre, Dominique Valteau-Couanet, Deborah A Tweddle, Ruth Ladenstein, Gudrun Schleiermacher (2021 Jun 11)
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 : JCO2100086 : DOI : 10.1200/JCO.21.00086

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 microcopy 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.

Eugenio de la Mora, Manuela Dezi, Aurélie Di Cicco, Joëlle Bigay, Romain Gautier, John Manzi,

Joël Polidori, Daniel Castaño Díez, Bruno Mesmin, Bruno Antonny, Daniel Lévy. (2021 Jun 7)
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.

Jingqi Dai, Aurore Sanchez, Céline Adam, Lepakshi Ranjha, Giordano Reginato, Pierre Chervy, Carine Tellier-Lebegue, Jessica Andreani, Raphaël Guérois, Virginie Ropars, Marie-Hélène Le Du, Laurent Maloisel, Emmanuelle Martini, Pierre Legrand, Aurélien Thureau, Petr Cejka, Valérie Borde, Jean-Baptiste Charbonnier (2021 Jun 5)
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.